

REPORT

GUIDELINE DOCUMENT FOR MEASURING THE BIOLOGICAL EFFECTS OF ACCIDENTAL OIL SPILLS

To:

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Glossary

Term	Definition
Alkanes	A family of compounds, also known as paraffins, that are saturated hydrocarbons with straight or branched chains, containing only carbon and hydrogen and have the general formula C_nH_{2n+2} ; generally from 5 to 40 carbon atoms per molecule.
Aromatic hydrocarbons (AH)	A family of compounds that have alternating double and single bonds between carbon atoms forming rings. The configuration of six carbon atoms in aromatic compounds is known as a benzene ring.
Asphaltenes	A group of hydrocarbon compounds that are characteristically very large and heavy relative to other hydrocarbons found in petroleum-based products. They do play a role in emulsification of oil in seawater. Asphaltenes are distinguished from resins by being insoluble in heptane.
Aryl hydrocarbon hydroxylase (AHH)	Aryl hydrocarbon hydroxylase, one of the families of mixed function oxidases. An enzyme found in vertebrates involved in metabolising hydrocarbon into metabolites or conjugates.
Batch Spill	A spill where the whole spill volume is effectively spilled within a very short period.
Biomarker	A measurable indicator within an organism of contaminant exposure.
Blowout	A discharge of oil, gas and water from the reservoir during a drilling operation. It is a continuous spill and can continue for an indefinite period.
BTEX (Benzene, toluene, ethylbenzene and xylene)	Collective term for a family of toxic, volatile, monoaromatic hydrocarbons.
DFO	Fisheries and Oceans Canada
Dilbit	Diluted bitumen
Ethoxyresorufin-O-deethylase (EROD)	Ethoxyresorufin-O-deethylase, one of the families of mixed function oxidases. An enzyme found in vertebrates involved in metabolising hydrocarbon into metabolites or conjugates.
ISB	In-situ burning
E&P	Oil and gas exploration and production
Hydrocarbon (HC)	An organic compound consisting entirely of carbon and hydrogen.
NCAG	National Contaminants Advisory Group
NOAA	National Oceanographic and Atmospheric Agency (US)
PAH	Polynuclear aromatic hydrocarbons are hydrocarbons with multiple aromatic rings.
Resins	A group of hydrocarbon compounds that are characteristically large and heavy relative to other hydrocarbons found in petroleum-based products. Resins are distinguished from asphaltenes by being miscible in heptane.
MFO enzymes	Mixed-function oxydase enzymes, important enzymes that catalyze detoxification of PAH using the cytochrome P-450 electron transfer system
Roundfish	Demersal species that are round in cross-section (e.g. cod, Pollock) as opposed to being flat (e.g., flatfish, plaice, halibut)
Sentinel species	Species that are already the subject of study in an existing monitoring plan

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1. Introduction

Activities of oil and gas industry pose a risk of oil spills into Canada's marine and freshwaters. These spills, in turn pose risks to environmental and human health, which we mitigate using countermeasures and restoration actions. During any spill, our response (countermeasures, restoration activities and planning) is guided, in part, by monitoring activities that are mounted during the spill, as well as by studies that have been conducted on previous spills. Oil spill monitoring is an invaluable tool for response and planning, but it is one of the most challenging of scientific endeavours. For that reason, many jurisdictions have developed plans (see Table 1) to guide the monitoring when needed and prepare in advance for implementing the plan. The present document provides information needed to plan for and conduct real-time spill monitoring of impacts of oil spills in Canadian waters on finfish, shellfish and mammals.

The impact of an oil spill on an aquatic ecosystem can vary widely depending on spill conditions (e.g., spill size, location, oil type, and season). Spill effects on individual fish can range from tissue contamination to mortality. Effects on populations can vary in magnitude, spatial extent and duration. Effects monitoring activities may have specific objectives to:

- a) Determine the severity of effects on individuals (contamination, sublethal effects, mortality);
- b) Determine the magnitude and spatial extent of impacts (e.g., Whitehead et al. 2014);
- c) Track the recovery of the population post-spill; and
- d) Link the observed impacts back to the spill itself.

The simple monitoring plan outlined in Section 4 of this report is designed to address each of these objectives using the most up-to-date techniques available at the time of writing. It is a step-by-step guide to monitoring strategies, tools and decisions during planning or real time response.

The potential effects of spills in Canadian waters are understood in a general sense from studies of earlier spills, most of which have occurred in other countries and climates. However, the behaviour and effects of spills depend heavily on a variety of factors (e.g., spill conditions, conditions of receiving environments). A large, northern country like Canada, with its broad ecological and industrial diversity, presents an extremely broad range of potential spill and environmental conditions, all of which will influence the spill's potential impact. In order to plan and respond to spills in Canada, it is critical to understand their behaviour and effects under Canadian conditions. For that reason it is critical to learn about our spills by monitoring them when they occur. In order to assist the monitoring team, this report contains an overview of the types of spill conditions potentially encountered in Canada in the coming years and the implications of each for monitoring. It also highlights some of the new features of spills (e.g., subsea blowouts, spills of dilbit) and countermeasures (subsea injection of dispersants). In addition, the report identifies knowledge gaps that might be addressed with future research in order to facilitate monitoring efforts.

This report is organized as follows. Section 2 provides the background for the work. Section 3 describes the types of spill scenarios that could potentially occur in the coming years, given the existing industrial setting. It also highlights new subjects that may not have been encountered by spill monitoring in the past. Section 4 contains a step-by-step guide to implementing a monitoring plan for a major spill, addressing steps in planning and decision-making. Section 5 provides a summary and recommendations

for specific areas of research and development that can improve our ability to mount monitoring efforts efficiently for spills in Canada.

2. Background

The impact of spills on fish, shellfish and marine mammals (hereafter referred to as fish) has been recognized for at least five decades, since at least the days of the Torrey Canyon spill (U.K., 1967; Zukerman 1967) and earlier. Although impacts of spills on fish may not always be as visible as impacts on marine birds, they are of concern, in part, because:

- a) Several species have commercial, subsistence and sport value to Canadians and;
- b) Several species of fish and marine mammals have migratory routes that extend across international borders and thus, are of international concern.

As a consequence, the effects of spills on fish has been investigated through studies of actual spills (e.g., Torrey Canyon, Amoco Cadiz, Prestige (see Table 2)), experimental spills (e.g., Baffin Island Oil Spill (BIOS) Project (Sergy and Blackall, 1987)) and experimental studies over the years (e.g., Anderson et al. 1974, Wells 1972, Gardiner et al. 2013). This work has been reviewed and summarized many times (e.g., for finfish and shellfish Trudel 1985, NRC 2003, Hylland 2006; for marine mammals Martin 1985, Geraci and St. Aubin 1988) and risk assessment models have been developed (e.g., French-McCay 2009) for use in planning and damage assessment. In the event of any spill of any significant size, it is critical to implement monitoring of fish and marine mammal populations as an integral part of the overall spill response, for several reasons as discussed below.

Oil spills have been shown to have a range of effects on fish including:

- a) Contamination of fish tissues by the spilled hydrocarbons (e.g., Edwards and White 1999, Law and Hellou 1999);
- b) Subtle reversible responses of fish to exposure to hydrocarbons at the biochemical and physiological level aimed at protecting the individual from effects of toxicants (Lee and Anderson, Crowe et al. 2013, Dubansky wet al. 2013) ;
- c) Less reversible injuries at the tissue, organ level to growth and reproduction (Marty et al. 2003, Giari et al. 2011); and
- d) Mortality (Schultz and Tebo 1975, Helton and Doty 2003, Michel et al. 1997).

The occurrence of injuries of these kinds to local fish populations are of interest to stakeholders, regulators and the public for a variety of reasons and these interests are addressed by monitoring. A few of the justifications for monitoring are listed in monitoring guides by Kirby et al. (2014) and ITOPF (no date) include the following:

- a) To assess the risk of transfer of hydrocarbons to human consumers;
- b) To provide early evidence of potential environmental and economic impact to key stakeholders (e.g. government and the general public);
- c) To determine the potential effects of the spill on commercial fish and shellfish to support decision-making regarding the need, or otherwise, to impose fishing restrictions;
- d) To provide monitoring and assessment input to the determination of compensation and/or liability issues as necessary;

- e) To establish whether or not any environmental effects observed are directly attributable to elevated oil concentrations arising from a particular spill event;
- f) To track the dissipation of contaminants in the environment and recovery of impacted biota;
- g) To provide an assessment of the effectiveness, or not, of spill response and clean-up activities, including the use of dispersants and in-situ burning;
- h) To aid in decision-making over the continuation or termination of the response; and
- i) To identify conditions appropriate for initiating and sustaining restoration measures.

The subject of environmental effects monitoring for spilled oil on fish has been reviewed by a number of authors. These publications identify a long list of biomarkers that could potentially be used for monitoring. However the review by Van der Roost et al. (2003) provides a simple system for organizing monitoring parameters that may be useful for the present oil spill monitoring application. Van der Roost et al. (2003) groups monitoring methods as follows:

- a) **Chemical monitoring (exposure monitoring):** assesses the exposure of biota to hydrocarbons in environmental compartments (water, sediments, etc.) by measuring hydrocarbon levels in abiotic environmental compartments;
- b) **Bioaccumulation monitoring:** assesses contaminant levels in biota;
- c) **Biological effect monitoring:** assesses the early adverse alterations that are partly or fully reversible (biomarkers); and
- d) **Health monitoring:** assesses the occurrence of irreversible diseases or tissue damage in organisms;
- e) **Ecosystem monitoring:** assesses the integrity of an ecosystem by making an inventory of, for instance, species composition, density and diversity. (This approach has seldom, if ever, been attempted during past spills.)

A number of monitoring guides have been published in recent years that provide guidance for monitoring a variety of aspects of effects of spills, including impacts on fish and marine mammals (see Table 1). These vary somewhat in their content and detail, but the more useful ones provide insight into the process of executing a plan. The more critical steps in executing a plan, after notification and description of spill conditions, include the following:

- a) Gather pre-existing information about baseline conditions;
- b) Complete field sampling to establish baseline conditions;
- c) Decide on purpose and specific objectives for monitoring;
- d) Develop a monitoring strategy;
- e) Develop a field sampling plan;
- f) Develop sample handling plan;
- g) Develop an analytical plan;
- h) Interpret data, communicate and use results and;
- i) Set terms for terminating monitoring.

These common elements of response plans have been incorporated into the plan presented in Section 4.

In short, the need for post-spill monitoring has long been recognized and considerable effort has been devoted to it during many past spills. Canada's natural and industrial environment poses a wide variety of potential spill and environmental conditions. This report attempts to provide:

- a) An overview of the potential spill and environmental risk conditions in Canada; and
- b) A simple generic plan for monitoring the effects of these spills, using up-to-date methods.

The document also includes an extensive list of references to studies and reviews of spill impact to introduce the user to the spill literature.

Table 1: Examples of Oil Spill Monitoring Guides, Plans and Methods

Australian Maritime Safety Authority. 2003b. Oil spill monitoring background paper. AMSA, Canberra.
Australian Maritime Safety Authority (AMSA). 2003a. Oil spill monitoring handbook. AMSA, Canberra
BC Ministry of the Environment. 2013. British Columbia Inland Oil Spill Response Plan. BC Ministry of the Environment.
Davies, I. M., and Vethaak, A.D. 2012. Integrated marine environmental monitoring of chemicals and their effects. ICES Cooperative Research Report No. 315. 277pp
IMO. 1998. IMO guidelines for sampling and identification of oil spills. Manual on oil pollution, Section VI. 44pp. London, UK. ISBN 978-92-801-1451-5
Kelly, C.A., Law, R.J. and Emerson, H.S. 2000. Methods of analysis for hydrocarbons and polycyclic aromatic hydrocarbons (PAH) in marine samples. Aquatic environment protection: analytical methods, 12. Cefas, Lowestoft, UK.
Law, R.J., Kirby, M.F., Moore, J., Barry, J., Sapp, M. and Balaam, J., 2011. PREMIAM – Pollution Response in Emergencies Marine Impact Assessment and Monitoring: Post-incident monitoring guidelines. Science Series Technical Report, Cefas, Lowestoft, 146: 164pp.
Martinez-Go'mez, C. et al. 2010. 2010. A guide to toxicity assessment and monitoring effects at lower levels of biological organization following marine oil spills in European waters. ICES Journal of Marine Science, 67: 1105–1118.
NOAA. 2003. The coastal resource coordinator's bioassessment manual. Office of Response and Restoration, National Oceanic and Atmospheric Administration. 260pp.
Robertson, S.B. 2001. Guidelines and methods for determining oil spill effects. Proceedings of the 2001 International Oil Spill Conference, pp. 1545–1548. American Petroleum Institute, Washington, DC, USA.
Wang, Z. and Stout, S.A. 2007. Oil spill environmental forensics: fingerprinting and source identification. Elsevier, Inc., Amsterdam, The Netherlands. 554pp. ISBN10: 0-12-369523-6. ISBN13: 978-0-12-369523-9.

Table 2: Examples of Historical Spills from Different Sources

Year	Name	Location	Type of Oil	Volume (tonnes)	Comments and References
Exploration/Production Spills					
2010	Deepwater Horizon	U.S., Gulf of Mexico	Light crude oil	780,000 m3 crude oil	Subsea blowout; the most thoroughly studied spill in history - Anonymous 2013
1979-80	Ixtoc - 1	Mexico, Bay of Campeche	Crude oil	480,000 m3	Largest oil spill of its day, first subsea blowout - Thibreau et al. 1981
1969	Santa Barbara Spill	U.S., California	Crude oil	16,000 m3 of crude oil	First major oil well blowout from offshore platform - Easton, 1972
1977	Ekofisk Bravo	North Sea, Norway	Crude oil	32,174	Mackie et al 1978
2009	Montara	Australia	Light, waxy crude oil	23,000	Platform blowout discharging as much as 320 m3 crude oil for 74 days, Monitoring study - Huang 2011
1984	Uniacke / Vinland	Canada, Scotian Shelf	Condensate	480	An extensive monitoring plan was put in place - Gill et al. 1985
2004	Terra Nova FPSO Spill	Canada, Grand Banks	Crude Oil	170	Wilhelm et al. 2007
Marine Cargo and Fuel Spills					
1993	Braer	UK, Scotland	Gulfaks Crude	84,700	The spilled oil was quickly dispersed by heavy winds - Perry 1995
1996	Sea Empress	UK	Forties	72,360	Chemical dispersants were a primary countermeasure - Edwards and White 1999
2002	Prestige	Spain	Bunker fuel oil	63,000	Heavy fuel oil - Fernández-Tajes et al 2011
1989	Exxon Valdez	U.S. Alaska	Alaska North Slope Crude	33,000	Heavy persistence of oil on shorelines; research into acute and long-term impacts - Weins et al. 1999
1991	Haven	Italy	Heavy Iranian crude	30,000	Effects of residue from in-situ Burning impacted fisheries - Martinelli et al. 1995
1996	North Cape	US, Rhode Island	Home Heating Oil	3100	Shallow, nearshore waters caused extensive mortality and contamination - Michel et al. 1997
2001	Jessica	Galapagos Is., Ecuador	No. 2 and Bunker	547	Unique types of impacts - Born et al. 2003
2006	Queen of the North	Canada, West Coast	Diesel and Light oil	243	Long term monitoring plan established
2007	Cosco Busan	U.S., San Francisco Bay, CA	Intermediate Fuel Oil IFO-380	203	Apparent impacts on herring populations in coastal zone
2004	Athos I Spill	U.S., Delaware R.	Venezuelan crude oil	113	Tankship spill
2007	Robson Bight Barge Incident	Canada, BC	Diesel	10	Inshore spill, no monitoring

3. Overview of Possible Spill Scenarios

Petroleum is spilled from a number of sources under a wide variety of spill conditions (e.g., type of product, spill size, duration, receiving environment). All of these influence the impact of the spill and the purpose of monitoring. The following describes some of the aspects of spill conditions that might influence monitoring activities.

3.1 Spill Sources

Accidental spills of petroleum into water may originate from any point in the petroleum exploration-production-refining-transportation-consumption chain. Transportation can include ships and barges, pipelines, rail and road tankers. Each spill source can produce a range of spill sizes, but the maximum possible spill size varies widely from source to source. For each source, spill probability declines with increasing spill size. Some statistics regarding spill frequency and size are listed in Table 3. In Canada, statistics for spills into the marine environment are readily available, but those for inland spills are not. Some statistics regarding spill frequency and size in Canada are listed in Table 3¹.

Table 3: Annual Frequency of Spills in Canada by Source and Volume

Source	Spill Volume (m ³)					
	<1	1-10	10-100	100-1000	1000-10000	>10,000
Offshore E&P^a	20.9	1.7	0.4	0	0	0
Vessel Spills^b						
Crude	nd	nd	0.02	0.01	0.19	0.004
Refined	nd	nd	0.6	0.10	0.024	0.00
Fuel	nd	nd	1.90	0.60	0.01	0.00
Total	nd	nd	2.52	0.71	0.05	0.004
Pipelines^c						
Release into water	0.56	0	0.2	0	0	0
Total	0.75	3.8	1.89	0.94	0	0
Rail	nd	nd	nd	nd	nd	nd
Road	nd	nd	nd	nd	nd	nd

Notes:

nd = no data. The designation nd was used to indicate information that was not publically available or immediately accessible.

a. 2004 to 2013 <http://www.cnsopb.ns.ca/environment/incident-reporting> and http://www.cnlopbnl.ca/env_stat.shtml

b. WSP Canada Inc., 2014.

c. 2008 to April 2013 - <http://www.neb-one.gc.ca/clf-si/rsftyndthnvrnmnt/sfty/pplnncdntgrprtng/pplnncdntshydrclbrnspills/qdr/ssbr-eng.html>

3.1.1 Exploration and Production (E&P)

E&P activities involve drilling deep wells into the earth in order to locate oil-bearing geological formations and extract the hydrocarbon liquids for refining and use. Often, the petroleum liquids in these formations exist under high pressures and can produce large accidental discharges of petroleum liquids if

¹ Different agencies in Canada are responsible for tracking spill statistics. The publication of data is not always consistent reported or readily available. Comparisons between sources should be interpreted with caution.

pressures are not effectively controlled. This can result in blowouts and include some of the largest spills in history, for example, the Gulf of Mexico spill in 2010 and the Montara spill in 2009 (Table 2). Oil spilled from E&P activities can range from very light, volatile, non-persistent oils (e.g., condensates) to heavy, viscous, persistent oils. One of the very few E&P spills in Canada's history, the Uniacke/Vinland blowout (1984), was a condensate spill (Gill et al. 1985). In Canada, at present, E&P activities that could result in blowout spills are ongoing on the east coast (e.g., Sable on the Scotian Shelf; Hibernia/Terra Nova/White Rose on the Newfoundland Grand Banks).

Special Considerations: Exploration and Production in the Arctic

In recent years, offshore oil E&P drilling in Canada has been restricted to temperate East coast waters. The Canadian Beaufort Sea was the site of considerable exploratory drilling in the 1970s and 1980s, but exploration was suspended for almost two decades, beginning in the late 1980s. In recent years however, interest in the Arctic was renewed. A new well was drilled at the Devon Paktoa C-60 site in the Beaufort Sea in 2006 (NEB/ONE 2006) and additional exploratory wells in the arctic are planned. To date, there have not been any major spills related to offshore oil exploration in Canada's Arctic, but should they occur, they could pose some challenges for monitoring. The reason for this is that the knowledge of the biology of key marine species (e.g., Arctic cod) is limited. In addition, Arctic marine ecosystems include unique habitats including, the epontic habitat². The epontic habitat does not occur in temperate waters and should be monitored in the event of a major Arctic spill. Research is ongoing concerning the ecology of fishes in Canadian Arctic waters and the epontic habitat. In preparation for monitoring, the results of Arctic marine research should be consolidated periodically to serve as a baseline for monitoring activities.

Special Considerations: Subsea Blowouts and Subsea Injection of Dispersants

In the past, most offshore drilling-related blowouts have involved oil discharges from platforms (i.e., above the sea surface) rather than from subsurface parts of the drilling system (i.e., below the sea surface). The recent blowout in the Gulf of Mexico in 2010 was unique in that oil was discharged under the sea and at great depth. From a monitoring perspective, subsea blowouts occurring in shallow water (i.e., at a few hundreds of metres or less) behave somewhat similarly to those at the surface and may be monitored in the same way. In these relatively shallow subsea blowouts, the gas discharged with the oil forms a bubble plume that carries the oil quickly and directly to the sea surface, where the oil forms slicks. Even if dispersants are injected into the subsurface plume, the bubble plume would be expected to push the dispersed oil cloud upward into surface waters. Once at the sea surface, the oil should behave similarly to oil dispersed from surface slicks.

Until relatively recently, all offshore oil exploration had been completed at relatively shallow depths (i.e., at a few hundreds of meters). However, in recent years, wells have been drilled in waters more than 1000 m deep. In deep water blowouts (i.e., >500 m deep), the hydrostatic pressure at the sea bed prevents gas bubbles from forming. Without gas bubbles, the subsea oil plume does not rapidly rise to the surface. Instead, the buoyant oil droplets drift upward at a much slower rate and arrive at the sea surface several hours or days after discharge, often at some distance from the spill site. Additionally,

² The epontic community is the biological community that thrives on the underside of the Arctic sea ice (seasonal ice, pack ice and land-fast ice). This community can contribute significantly to the energetics of the Arctic marine ecosystem.

during the Gulf of Mexico spill in 2010, chemical dispersants were injected at the deep subsea blowout site. From a monitoring perspective, subsea dispersant injection into these deep subsea blowouts poses a significant challenge. During the Gulf of Mexico spill, chemical dispersants injected at the deep subsea blowout site caused the formation of much smaller oil droplets at the discharge site relative to undispersed oil. As a result, these droplets very slowly rose to the surface and were transported horizontally over very long distances from the spill site. Most of these droplets did not surface at all, but rather formed a subsea plume of oil being carried away long distances from the spill site by deep currents. This scenario can cause significant challenges to monitoring teams, as resources are utilized to locate and track the subsurface oil plume. Under these conditions, monitoring of immediate biological effects caused by the spill may be difficult if the subsurface oil plume is not well characterized.

3.1.2 Petroleum Transport

In general, crude oil is transported from where it is produced to refineries where it is refined and then transported to consumers. This involves a variety of modes of transport. Transportation of crude oil and refined products to and from refineries often consists of transport by tanker ships, pipelines and trains. Meanwhile, transport of refined product from local distributors to retailers and consumers is often accomplished with tanker trucks. Table 2 contains several examples of spill accidents involving transportation methods in Canada and worldwide. Meanwhile, Table 3 gives an overview of annual spill frequency in Canada by transport method.

Spills from Tanker Trucks

Tank trucks generally deliver small quantities of refined fuel from storage depots to the consumers. Spills from tank trucks generally involve relatively small amounts (up to 35-40 m³) of non-persistent refined oil. In a few situations, spilled products have entered into freshwater rivers or lakes (e.g., Lemon Creek spill, B.C.) (Table 2). The largest of these spills have had significant acute effects in the past and have required post-event monitoring. When large truck spills enter a small river or lake with limited dilution potential, the spill can cause high concentrations of hydrocarbons in receiving waters. This can produce significant fish kills in localized areas and cause environmental contamination for long distances downstream from the spill. Monitoring may be required to manage health and safety risks for nearby human population and impacts on local fish and wildlife populations. This is discussed more fully in the "Receiving Environments" section of this report (Section 3.4). Spill response measures for these spills will involve mechanical cleanup and shoreline cleaning. Since these spills most commonly involve non-persistent oils, evidence of contamination and effects may be relatively short-lived in the pelagic and riverbed environments, although hydrocarbons may persist for longer in shoreline sediments.

Spills from Rail Tank Cars

Rail tank cars with a capacity of approximately 131 m³ are used to transport both refined fuels and crude oils in Canada (Figure 1). Spills originating from rail tank cars can be larger than those from the tanker truck spills and depend on the number of cars involved (e.g., historical spills such as Lake Waubamun and Lac Megantic have involved 750 and 4830 m³) (Table 2). Rail-related accidents can involve persistent or non-persistent oils spilled into freshwater lakes and rivers. These spills will involve mechanical cleanup and shoreline cleaning.

In some situations, spills from rail tank cars can enter inland waters, leading to severe impacts extending long distances from the spill site and requiring monitoring. Monitoring may be required to manage the health and safety risks to the nearby human population and/or for measuring impacts on local fish and wildlife populations. For example, the train derailment and spill of Bunker C fuel oil into Lake Waubamun, Alberta, in 2005 involved post-event monitoring for indicators of reproductive success in local fish species (Debruyn et al., 2007). That incident involved measuring levels of environmental contamination and in-situ testing to determine if existing levels of contamination could impact native fish species. Further considerations for monitoring the effects of spills in rivers and lakes are discussed in the section on “Receiving Environments” of this report (Section 3.4).



Figure 1: Rail Car Used for Transporting Bulk Petroleum

Spills from Pipelines

Pipelines are used to transport crude oil from production sites to refineries and to transport refined oil from refineries to distribution centres. Most of the crude oil pipelines are inland, but there is at least one offshore pipeline in Canada, the Sable subsea pipeline, extending from the Sable Offshore Energy Project near Sable Island to the Goldboro Gas Plant in Guysborough County, Nova Scotia.

Pipeline spills can involve discharges of persistent or non-persistent oil into marine and freshwater environments. Spills are commonly of intermediate volume (e.g., from a few 10s to a few 1000s m³ of oil) (Table 3). In Canada, small inland pipeline spills have been relatively common in recent years (see Table 3), though few of these have resulted in oil reaching freshwater or marine environments. Spills into freshwater and marine water will involve mechanical cleanup and shoreline cleaning, while spills into the marine environment may also include the use of dispersants and in-situ burning. Some of the spills, especially those reaching rivers and lakes, will require monitoring to track health risks to human users and risk of injury and contamination of any commercial or recreational fish stocks.

Spills of Cargo from Tanker Ships and Barges

Spills of cargo from tanker ships or barges are the most diverse group in terms of possible spill conditions. They may be small or very large in size and can involve persistent or non-persistent oil. Environments where these types of spills can occur include marine (e.g., offshore, coastal), freshwater (e.g., Great Lakes) or estuarine environments (e.g., St. Lawrence River). Spills in rivers may involve mechanical and shoreline cleanup, while those at sea may involve dispersants and burning as well. Spills of non-persistent oils in deep, offshore, environments may not involve cleanup activities.

Most nearshore or inland spills of any size may require monitoring impact monitoring for purposes of post-incident litigation and compensation. In deep, offshore waters, large spills require some monitoring, especially if they threaten areas that support exploited fish stocks. Spills of non-persistent oils

In some situations, spills from rail tank cars can enter inland waters, leading to severe impacts extending long distances from the spill site and requiring monitoring. Monitoring may be required to manage the health and safety risks to the nearby human population and/or for measuring impacts on local fish and wildlife populations. For example, the train derailment and spill of Bunker C fuel oil into Lake Waubamun, Alberta, in 2005 involved post-event monitoring for indicators of reproductive success in local fish species (Debruyne et al., 2007). That incident involved measuring levels of environmental contamination and in-situ testing to determine if existing levels of contamination could impact native fish species. Further considerations for monitoring the effects of spills in rivers and lakes are discussed in the section on "Receiving Environments" of this report (Section 3.4).



Figure 1: Rail Car Used for Transporting Bulk Petroleum

Spills from Pipelines

Pipelines are used to transport crude oil from production sites to refineries and to transport refined oil from refineries to distribution centres. Most of the crude oil pipelines are inland, but there is at least one offshore pipeline in Canada, the Sable subsea pipeline, extending from the Sable Offshore Energy Project near Sable Island to the Goldboro Gas Plant in Guysborough County, Nova Scotia.

Pipeline spills can involve discharges of persistent or non-persistent oil into marine and freshwater environments. Spills are commonly of intermediate volume (e.g., from a few 10s to a few 1000s m³ of oil) (Table 3). In Canada, small inland pipeline spills have been relatively common in recent years (see Table 3), though few of these have resulted in oil reaching freshwater or marine environments. Spills into freshwater and marine water will involve mechanical cleanup and shoreline cleaning, while spills into the marine environment may also include the use of dispersants and in-situ burning. Some of the spills, especially those reaching rivers and lakes, will require monitoring to track health risks to human users and risk of injury and contamination of any commercial or recreational fish stocks.

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and smaller spills of persistent oils may not require monitoring, as they may dissipate quickly and pose limited threat to water column and seabed species.

3.2 Oil Types

Petroleum liquids are a diverse assemblage of materials that include a) crude oils (crudes), with a broad range of compositions and properties, and b) refined petroleum products (products) derived from crude oils, that have a similarly broad range of properties. Each crude and product contains from dozens to hundreds of different hydrocarbon compounds spanning a broad range of molecular sizes, densities, volatilities and toxicities. Light crude and refined oils are composed mostly of low molecular weight hydrocarbons (such as butane) and are generally more volatile (e.g., gasoline or natural gas condensate) (Table 4). On the other hand, heavy crude and some residual fuel oils are composed of greater amounts of high molecular weight hydrocarbons such as resins and asphaltenes that are generally less volatile (e.g., bunker C, bitumen) (Table 5). Examples of Canadian crude oils include condensates (e.g., Sable and Panuke), conventional crude oil (e.g., Terra Nova, Norman Wells) and heavy to extra heavy crude oil (e.g., bitumen from Cold Lake and Athabasca oil sands deposits) (Table 5).

3.2.1 Special Considerations: Aromatic Hydrocarbons, Persistence and Fingerprinting

From the perspective of monitoring, several aspects of the product's composition and properties are important to consider: a) the identity and relative composition of aromatic hydrocarbons, b) the persistence of the substance following a spill into the environment and, c) the identity of fingerprinting biomarkers present in the oil.

Aromatic Hydrocarbons

Aromatic hydrocarbons, particularly the polycyclic aromatic compounds (PAH) (e.g., naphthalene, phenanthrene, benzopyrene), have been shown to cause toxicity to aquatic biota. All crude oils and most products contain some PAH and hence, pose a risk of causing lethal and sublethal toxicity to aquatic biota should a spill occur. It is most commonly the interactions of PAH with organisms systems that produce the responses that are measured in monitoring.

Persistence of Oil

Persistence of a petroleum substance is defined as the length of time that components of the oil remain in the environment following a spill. Persistence of oil components can be related to those found in surface slicks, water-column, shorelines and benthic sediments. Some crudes or products dissipate quickly (within hours or a few days), while others can persist for many years in some environmental compartments. In this context, persistence of any type of oil depends mostly on the relative proportion of volatile to non-volatile components in its composition. Generally, non-persistent oils are those with greater proportions of volatile components (e.g., gasoline, natural gas condensates) (Tables 4 and 5). By contrast, persistent oils and products are those that contain a significant proportion of high-molecular weight compounds, including asphaltenes and resins. These compounds tend to persist as they are generally not volatile and resist microbial degradation. Most crude oils (e.g., Hibernia, Terra Nova and White Rose crude oils) and a few refined products are composed of mixtures of both persistent and non-persistent components.

The internationally accepted technical guideline for assessing persistence of oil is as follows: "...non-persistent oil is oil which, at the time of shipment, consists of hydrocarbon fractions, (i) at least 50% of which, by volume, distills at a temperature of 340 °C (645°F) and (ii) at least 95% of which, by volume, distills at a temperature of 370 °C (700°F); when tested by the ASTM Method D86/78 or any subsequent revision thereof..." (Anderson 2001) (see Appendix 1). This guideline was used to identify persistent versus non-persistent crudes and products in Table 5.

Fingerprinting Biomarkers

Fingerprinting biomarkers are specific persistent hydrocarbon compounds that are present in most oils in unique composition and levels and hence are useful for oil identification purposes or "fingerprinting". Most components of oil undergo changes in chemical composition quickly when spilled due to biodegradation and weathering. However, these biomarker compounds resist these processes and can be used to link the spilled oil to the original oil source extracted from the environment. These biomarkers include: pristane, phytane, steranes, triterpanes and porphyrins. Note that the term "biomarker", used here for purposes of oil fingerprinting should not be confused with its usage in describing groups of indicators of biological effects.

3.2.2 Diluted Bitumen

Diluted bitumen is a blend of bitumen and a diluent (e.g., natural gas condensate) made for the purpose of meeting pipeline viscosity and density specifications. It is commonly referred to as "dilbit". The density of Alberta's oil sands bitumen is approximately 1.01 g/cm³ and, when diluted with condensate with a density of approximately 0.80 g/cm³ this yields dilbit with a density of approximately 0.925 g/cm³. Dilbit is highlighted here because of its complex and less well understood fate when spilled in water. When spilled into saltwater, dilbit may behave like very heavy crude oil, floating and creating surface slicks. On the other hand, when spilled into freshwater, the oil may float initially, but sink after the light ends have been lost by evaporation. This behaviour is common for other heavy crude oils. Diluted bitumen represents a major component of Western Canada's oil production and its production is projected to exceed that of conventional crude oil in Alberta in the coming years (CAPP 2013).

Table 2: Examples of Products of Fractional Distillation of Crude Oil

Product	Molecular Size (as # of carbons)	Density (kg L⁻¹)	Comments
Light Distillate			
Gasoline	C4-C12	0.71-0.77	Automobile fuel
Naphtas	C6-C12	0.75-0.79	Feedstock for gasoline, petrochemicals, diluent for bitumen
Middle Distillate			
Kerosene	C6-C16	0.78-0.81	Component of jet fuel
Diesel Fuel	C8-C21	0.83-0.85	Automobile fuel, 75% alkanes, 25% aromatics
Residential Heating Fuel (No.2 Fuel oil)	C14-C20	0.86-0.87	Home heating oil
Heavy Distillate			
Heavy fuels / Bunker / Residual fuels	C12-C70	>0.87	Bunker C, No.5 or 6 fuel oil
Residue			
Asphalt	Alkanes >C40		Solid residue remaining after distillation of crude oil; large stable non-volatile

Table 3: Examples of Non-Persistent and Persistent Crude Oils and Refined Products

Oil Type	Boiling Point for Fraction ^{a,b} , °C		Density ^b , kg L ⁻¹ (15 C°)	Comment
	50% ^c	95% ^d		
REFINED PRODUCTS				
Aviation Gasoline	100	125	0.72	non-persistent
No. 1 Fuel Oil	130	225	0.75-0.84	non-persistent
Automotive Diesel	225	275	0.83	non-persistent
No 2 Fuel Oil	260	370	0.86	non-persistent
Threshold for Non-Persistent Oil	340	370		Threshold
IFO 30	460	675	0.914	persistent
IFO 180	525	750	0.951	persistent
Bunker C	600	700	0.97-0.99	persistent
Orimulsion	600	>750	1.01	persistent
CRUDE OILS				
Cold Lake Diluent	60	150	0.716	non-persistent
Sable Condensate ^e	100	225	0.741	non-persistent
Panuke Condensate	175	350	0.787	non-persistent
Threshold for Non-Persistent Oil	340	370		Threshold
Amauligak	300	550	0.901	persistent
Alberta Sweet Mixed Blend	300	625	0.861	persistent
Norman Wells	300	625	0.845	persistent
Terra Nova	325	650	0.871	persistent
Alaska North Slope	300	725	0.877	persistent
Transmountain Blend	350	650	0.865	persistent
Cold Lake Blend	475	>725	0.927	persistent
Cold Lake Bitumen	575	>>750	1.0075	persistent

Notes:

- From Andersen (no date)
- Data from Environment Canada Oil Properties Database see <http://www.etc-cte.ec.gc.ca/databases/OilProperties/>
- Temperature below which 50% evaporates
- Temperature below which 95% evaporates

3.3 Spill Countermeasures

Spill countermeasures are techniques used to treat spilled oil to reduce its environmental impact and speed ecosystem recovery. For most spills of oil into water, the most injurious feature of the spill is caused by the formation of surface slicks that can persist for days to weeks and, in many cases, strand on shorelines. Countermeasures are sometimes used to treat oil in water but also on shorelines. On-water countermeasures include mechanical recovery, in-situ burning and the use of chemical dispersants. Onshore treatment involves a variety of techniques that seek to remove the oil from the shore without unduly disturbing the shoreline substrate (e.g., collection, washing, tilling, etc.). The impact and monitoring implications of these countermeasure methods are discussed below.

3.3.1 On-Water Countermeasures

Mechanical Recovery

Mechanical containment and recovery involves collecting oil slicks using a towed floating boom, picking up the oil using skimmers and transporting the oil to shore for disposal. Figure 2 shows collection booms at sea (a) and on a river (b). Skimmers can also be used to remove oil from the surface (Figure 2b). Mechanical recovery has few environmental side effects that have implications for monitoring.

In-Situ Burning

In-situ burning (ISB) involves collecting slicks using floating booms (as with mechanical recovery) and then igniting and burning the oil in the boom, rather than picking it up with a skimmer (Figure 3a). After the burn extinguishes itself, a small amount of semi-solid, non-toxic hydrocarbon residue remains and is released. The burn operation, smoke plume and, in most cases, the burn residue poses little to no risk to fish or marine mammals. The exception is when heavier oils are burned, producing a dense residue that sinks when it cools. Sunken residue has disrupted commercial fisheries in the past when pieces of residue were collected in fishing nets along with fish (Martinelli et al., 1995). The residue itself appears to pose little toxic or contamination risk to fish or shellfish because most of the PAHs are driven off by the burn and remaining components are not bioavailable. However, experience with actual burning operations and residue is very limited. For that reason, burning operations should be monitored to track the fate of the residue. If residue sinks, fish and shellfish from contaminated habitats should be monitored for evidence of exposure to PAHs.

In general, ISB is only considered for offshore marine spills or for inland spills in remote locations because of the human health issues associated with the smoke plume generated by the fire.

Chemical Dispersants

Dispersants are specialized chemicals used to disperse oil slicks from the sea surface into the water column (Figure 3b). Dispersion dissipates oil slicks, but generates clouds of tiny oil particles (< 70 µm in diameter) in the upper few metres of the water column. Initially, oil concentrations in these clouds are high enough to cause toxicity and contamination in fish, but concentrations decline quickly (i.e., within hours) to background levels by dilution. There is little evidence that chemical dispersion of oil directly impacts marine mammals. In general, the small oil droplets formed when chemically dispersed aid in enhancing degradation (e.g., via bacterial degradation). Some oil droplets can also bind to inorganic or organic sediments found in the water column and subsequently sink to the sea bottom.

Dispersant operations are still relatively new in North America and for that reason, when dispersants are used, monitoring should include measures of hydrocarbon contamination and effects on both benthic and pelagic aquatic biota.

Currently, chemical dispersants are only used when oil is spilled in saltwater because most commercially-available dispersant products are ineffective in freshwater.

3.3.2 Onshore Countermeasures

Shoreline Cleanup

Shoreline cleanup involves removing oil that has become stranded using a variety of methods (e.g., manual collection, flushing, tilling) (Figure 4a, b). The objective is to remove the oil from the shore in order to minimize its impact and reduce its persistence. One challenge is that some cleaning methods can damage shorelines and shoreline biota. For that reason, responders balance the need to use methods that are effective for oil removal but that neither excessively damage local biota nor increase the persistence of the remaining oil. With certain methods (e.g., flushing) some of the oil washed off shorelines can disperse in nearshore waters and settle in subtidal areas where fish can become exposed to toxic components. For this reason, it may be useful to monitor subtidal areas adjacent to shoreline cleanup operations to test for contamination of sediments and fish exposure to hydrocarbons.

3.3.3 New Countermeasures

Subsea Dispersant Injection

As discussed in Section 3.1, most offshore blowouts involve oil discharged above the sea surface. However, there are occasions when accidental discharges occur below the surface of the sea (i.e., subsea). When this happened in the Gulf of Mexico Spill, in 2010, operators quickly developed a new response technique that involved injecting dispersants into the oil plume at the point of discharge. This technique was termed “subsea dispersant injection” (or “subsea injection”) and it is likely to become standard practice for any future subsea discharges.

Subsea injection involves using a hose to inject dispersant directly into the blowout plume as it exits the drill pipe. The effect of the dispersant is to cause the oil to be sheared into very tiny droplets. In shallow water blowouts the resultant dispersed oil cloud would be propelled to the surface by the rising plume of gas bubbles, surfacing at the spill site. In deep water blowouts, a gas bubble plume does not form and thus, oil droplets ascend to the surface at a very slow rate. As a result, the oil droplets can drift horizontally and surface many kilometers away from the spill site. It is also possible that some of the oil droplets fail to surface and remain in the horizontal subsea plume and/or eventually settle to the sea floor. In incidents where subsurface oil plumes are possible, the objective of monitoring will be to track the oil plume and the resulting seabed contamination to assess exposure of finfish and shellfish species to oil and to determine the resulting impacts.

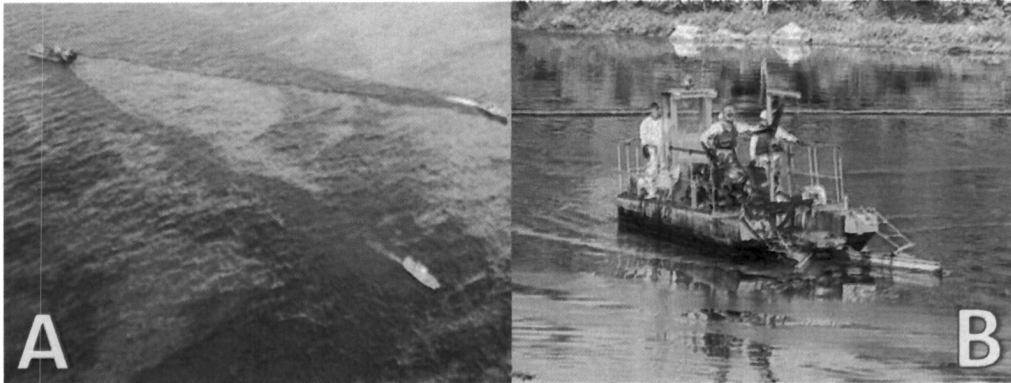


Figure 2: Mechanical Collection of Oil: At Sea (A); On A River (B)

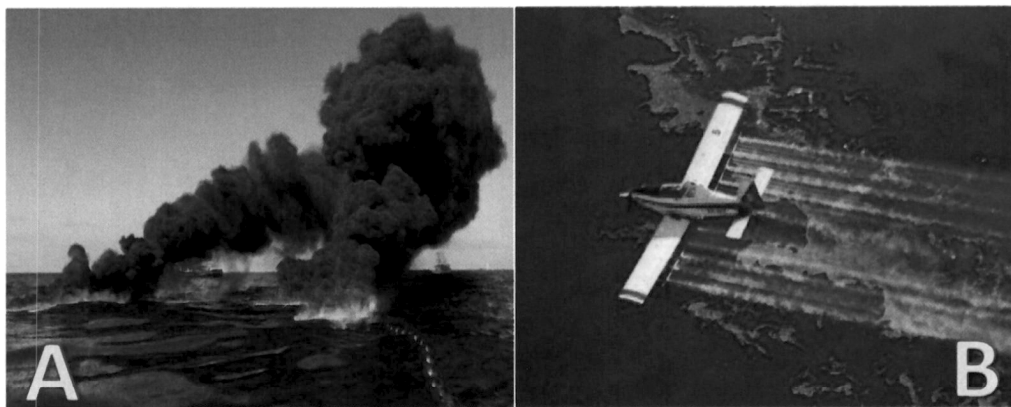


Figure 3: On-Water Countermeasures: In-Situ Burning (A); Dispersants (B)



Figure 4: Shoreline Cleaning: Manual (A); Washing (B)

3.4 Receiving Environments

Receiving environments refer to the types of aquatic environments where oils are spilled. This includes: deep offshore marine waters, shallow nearshore marine waters and inland rivers and lakes. Each environment can receive small or large spills of persistent and non-persistent oils from some or all of the potential sources. The fate and impact of spills into each are briefly described here, as are the potential needs for monitoring.

3.4.1 Deep Offshore Areas

This refers to marine spills into deep waters, many kilometers from shore. In general, spills into deep, offshore marine areas can originate from drilling platforms, tankers, barges or pipelines. They may involve persistent or non-persistent oils. Generally, these spills form surface slicks that spread and dissipate causing minimal toxicity to biota in the water-column because the dispersion into the water-column does not occur quickly. A portion of the oil may become entrained into the water over time and ultimately settle to the seabed. However, this will happen slowly after the oil has weathered for some days on the sea surface. Weathering will degrade the oil meanwhile prevailing conditions at the sea surface can disperse the oil over a broad area. As a result, the eventual dispersion of oil into the water-column and sinking to the seabed would cause low levels of contamination in any given area. The extent of dispersion generally depends on wind conditions and sea state. Fish/shellfish mortalities and high levels of oil contamination in the water column or sediments are not likely.

The objectives of monitoring might be to determine:

- a) If measurable levels of seabed contamination have occurred;
- b) If benthic species have been exposed to oil and have suffered tissue contamination and sublethal effects;
- c) The spatial extent of spill-related contamination;
- d) The persistence of contamination; and
- e) The linkage between the spill and any observed impacts.

In general, spills of non-persistent oils and/or small spills of persistent oils probably will not be candidates for more than basic monitoring (e.g., identifying presence/absence of seabed/water column contamination and exposure of fishery species).

There are a few situations where a more extensive monitoring program should be implemented. These exceptions are blowout spills or spills involving dispersants or ISB.

Blowout Spills

All blowout spills require monitoring because very large amounts of oil may be involved. Of particular interest are subsea blowouts in waters deeper than 500 m (e.g., the Gulf of Mexico spill in 2010), because these can form subsea plumes of oil. The behaviour of these subsea plumes is still poorly understood and will require extensive monitoring for all fate and effects parameters.

Dispersants

Spills involving dispersants may result in large amounts of relatively unweathered oil entering the water-column and potentially causing sedimentation of oil to the seabed. There should be little risk of fish mortality in the water-column, but there is possibility of contamination of the water-column and seabed. Risks to pelagic species are low because of greater dilution potential in offshore regions. Fish tissue contamination may occur, but is likely to be transient. Monitoring should track contamination of habitats, water-column and seabed species.

In-situ burning

In-situ burning poses little to no risk to water-column species. However, if heavier oils are burned (e.g., with a specific gravity of fresh oil > 0.85 to 0.88 gm/cm^3) some residues may sink (Buist et al. 1997). Evidence shows that the burn residues pose no acute toxic risk to seabed species (Blenkinsopp et al. 1994, Gulec and Holdway 1999). However, sunken residues may foul fishing gear if in direct contact at the seabed. Recognize, however, that experience with sinking residue is still limited and for that reason, some monitoring of benthic fish for exposure to PAH would be recommended in spills involving ISB until more experience is gained.

3.4.2 Nearshore Marine (<30 m deep or <20 km offshore)

Spills into shallow or nearshore waters can result from surface blowouts from exploration or production activities, tankers/barges or pipelines (e.g., the Sable offshore gas/condensate pipeline). These spills will form slicks, spread, and dissipate, similar to spills in offshore regions. In contrast to offshore spills, some oil may strand on nearby shorelines and will become entrained into the water-column exposing pelagic and benthic species to oil. In addition, entrainment can lead to significant contamination of the seabed (e.g., Braer, North Cape Spills) (Table 1). Also, some of the oil stranded on shorelines may become redistributed into shallow nearshore waters causing longer-term contamination. In short, hydrocarbon contamination of the water-column, shoreline and seabed are likely. Contamination and mortalities of fish/shellfish in the water-column and on the seabed are possible, depending on conditions. Impacts and contamination of nearshore marine mammal species are also possible.

Monitoring should include:

- a) Sampling of seabed sediments, water column and shoreline sediments for hydrocarbon contamination (presence, spatial extent and duration) and fingerprinting;
- b) Collecting deceased fish/shellfish and marine mammals for enumeration, necropsy, tissue contamination and hydrocarbon fingerprinting;
- c) Sampling living fish and shellfish for contamination, spatial extent and duration of contamination and fingerprinting; and
- d) If contamination is positive, analyse fish/shellfish for sublethal effects (spatial extent, duration and correlation with contamination).

Special consideration should be given to spills involving the use of dispersants in nearshore waters. These spills will have a high likelihood of fresh oil entering the water-column exposing pelagic and benthic species to oil. There is potential for fish contamination and mortality.

3.4.3 Freshwater Rivers/Lakes

In general, spills into rivers and lakes may originate from ships/barges, pipelines, trains and trucks. Most oils will float at least initially but turbulence and potentially high sediment load can cause oil to mix quickly into the water-column. As a result, this can cause contamination of water and sediments and mortality/sublethal effects in fish and shellfish. In small lakes, rivers and streams spills can result in significant impacts and contamination given the low volume of water and thus, decreased potential for dilution of spilled products.

Monitoring should include:

- a) Quantification of fish mortality;
- b) Determination of habitat contamination by hydrocarbons including measures of concentrations, spatial extent and persistence. This may be useful in linking observed effects to spill;
- c) Determination of contamination of biota by hydrocarbons including: measuring concentrations in tissues, determining the spatial extent of affected biota, and tracking persistence;
- d) Determination sublethal effects in biota to hydrocarbons including: measuring indicators of sublethal effects, determining the spatial extent of affected biota, and tracking persistence and;
- e) Establish linkage between the oil spill and the monitoring measures in a) to d).

4. Guide for Effects Monitoring of Fish during Oil Spills

This section contains a step-by-step guide for monitoring the biological effects of an accidental petroleum spill on fish, shellfish and, to some extent, marine mammals in Canada. The steps are listed in Table 6. Brief explanations of each step are provided for each in the paragraphs that follow.

Table 4: Steps in the Monitoring Plan

A.	Receive notification of a spill incident and acquire information about the spill
B.	Make a preliminary assessment of the location/size of the area potentially contaminated
C.	Identify sources of baseline biological data for the receiving environment;
D.	Decide if there is a need for field monitoring activity
E.	Coordinate with Other Monitoring Programs Operating for this Incident
F.	Conduct initial and subsequent spill site area reconnaissance
G.	Identify the monitoring objectives
H.	Identify the monitoring species
I.	Identify the effects (biomarkers) to be monitored
J.	Identify the monitoring strategy
K.	Identify the spatial scope of monitoring and identify control
L.	Identify the effects (biomarkers) to be monitored
M.	Identify the spatial scope of monitoring activity and identify control sites
N.	Establish criteria for terminating monitoring activities
O.	Develop formal monitoring plan
P.	Implement plan
Q.	Reassess plan periodically
R.	Communicate Monitoring Results
S.	Terminate Monitoring

4.1 Step-by-Step Plan

The following provides some background and guidance in implementing each step of the plan.

A. Receive notification of a spill incident and acquire information about the spill

The initial spill notification and subsequent spill-related communication provides the information required to make an initial assessment of monitoring needs (e.g., spill location, source, volume, oil type, spill conditions). In Canada, the national and regional emergency spill reporting contacts of Environment Canada's Environmental Emergencies Program are available to receive reports of environmental emergencies, including oil spills (<http://www.ec.gc.ca/ee-ue/default.asp?lang=En&n=869D7A30-1>). In some regions, arrangements have been made to consolidate spill reporting as a "one-window" harmonized system. Arrangements are in place in the Atlantic Region and also, in Ontario, Alberta, Saskatchewan, Manitoba, and the Northwest Territories. This arrangement is soon to be extended to include British Columbia and Nunavut.

The regional environmental emergencies officers (EEO) are responsible for obtaining sufficient information to assess the situation, initiating appropriate follow-up, advising the regional environmental emergencies coordinator and senior departmental management when necessary, and ensuring that a regional fan-out has occurred. Procedures are set out in the Standard Operating Procedures for Environment Canada's Environmental Emergencies Officers. The spill information initially reported includes items in Table 7.

Table 5: Information Required in Initial Spill Notification

- | |
|---|
| <ol style="list-style-type: none">1. Date and time of the occurrence or observation of the spill, and the report to EEO2. Name and organization of observer and/or caller, contact number3. Substance spilled (if unknown describe appearance, odour)4. Estimated quantity spilled (basis for estimate);5. Location of spill6. Polluter and/or source of spill7. Affected environment (marine, land, etc.)8. Local weather/atmospheric conditions9. Apparent consequences (fish kill, spill contained, evacuation, etc.)10. Actions being taken to control spill11. Agencies notified or on-scene12. Safety concerns13. Other information |
|---|

B. Assess the location and size of the area contaminated by the spill incident.

In order to make a rapid preliminary assessment of the potential impact of the incident it is recommended to use available spill fate/trajectory modeling (See examples in Table 8) to estimate the size and location of the areas potentially contaminated by oil from this spill.

In general, there are three types of scenarios:

- a) For offshore open-water spills (marine or freshwater), oil slicks can be transported in any direction by the combined influences of winds and water currents. Use a suitable oil fate model to estimate the persistence and spreading of the spill aided by more basic approaches using maps and compass to estimate possible directions of spill movement. The likelihood of significant seabed contamination will depend on water depth and the type of spill (i.e., surface spill, subsea spill).
- b) For near shore open-water spills, at least a portion of the oil will be transported along shore, deposited on shorelines and contaminate subtidal and offshore sediments. More sophisticated fate and trajectory models will be required for these calculations due to the added complexity of near shore influences.
- c) For spills into confined waters, such as spills in rivers, oil will be transported downstream and contaminate the river banks and also mix into the water-column. The key concerns are: the distance traveled by the oil slick downstream; the possibility that entrained oil exceeds background concentrations and; the possibility of contaminating of the bottom sediments. Regrettably, at present no off-the-shelf computer model system is available for river spills, although several authors have published theoretical models in the past (e.g., Gogoase-Nistoran et al. 2008, Sayed et al. no date, Sheh et al. 1993). For that reason, it may be useful to develop a simple generic oil fate model for rivers to assist in decision-making and planning.

Recognize that this type of assessment is of preliminary nature and should be used for planning purposes only. If needed, more precise estimates of oil fate and environmental damage can be developed as the spill proceeds, using modeling tools (e.g., SIMAP) (see Table 8) and more accurate environmental data (e.g., water current fields).

Table 6: Examples of Software for Estimating Fate and Movement of Oil Spills

Oil Fate Models

- ADIOS2 (NOAA)^a – is an oil weathering model that incorporates a database containing more than a thousand crude oils and refined products, and provides quick estimates of the expected characteristics and behavior of oil spilled into the marine environment.

Trajectory and Integrated Models

- SLROSM^b – an oil spill model with strengths in oil behaviour at source and oil property change estimation.
- OILMAP (RPS ASA)^c – provides rapid predictions of the movement (trajectory) and persistence of spilled oil
- SIMAP (RPS ASA)^c – provides detailed predictions of the three-dimensional trajectory, fate, biological effects, and other impacts of spilled oil and fuels.

Notes:

- a. <http://response.restoration.noaa.gov/oil-and-chemical-spills/oil-spills/response-tools/downloading-installing-and-running-adios.html>
- b. http://www.slross.com/publications/SLR/Description_of_SLROSM.pdf
- c. <http://www.asascience.com/software/simap/index.shtml>

C. Identify sources of baseline biological data for the environment near the spill site

The objective here is to assemble information about the species present in the area at risk from the spill. This will provide information on: presence of species, abundance of species, species of economic and cultural importance, sensitive species and species under some form of designation under the Species at Risk legislation. This will aid in planning and decision-making for monitoring. Information can be gleaned from published sources or by consultation with local experts.

The required information includes the following:

- a) Species of potential interest in the area threatened by the spill (e.g., sentinel species, most abundant species, protected species, exploited species (i.e., subsistence, commercial, recreational));
- b) Abundance and spatial distribution of key species within and beyond the areas threatened by oil;
- c) Location of nearby areas that could serve as uncontaminated control areas for comparative purposes (e.g., similar habitat, same species, age distribution, conditions, physical conditions); and
- d) Information concerning baseline levels of biomarkers in key species prior to the spill (e.g., tissue levels of PAHs, EROD levels, information on spatial or seasonal variation in biomarker parameters).

In some cases, this information may be available from existing sources. The first source of basic species information will be Environment Canada's Environmental Sensitivity maps, which can be accessed through the Environmental Emergencies Coordination Centre (See Contact Information in box below). In some locations where industrial development has taken place, project-specific Environmental Impact Statements (EIS) may be available or descriptive studies of the local environment can be accessed

(Stantec, 2013). Alternatively, it may be necessary to rely on local resource experts to mount a field sampling project of obtain the information.

<p style="text-align: center;">Contact Information:</p> <p style="text-align: center;">Environmental Sensitivity Mapping Environment Canada Environmental Emergencies Coordination Centre, Program Development and Innovation (Montreal)</p> <p style="text-align: center;">Key contact for maps: Stéphane Leblanc Manager stephane.leblanc@ec.gc.ca 514-283-2337</p> <p style="text-align: center;">Or</p> <p style="text-align: center;">Regional contacts: https://cepae2- lcpeue.ec.gc.ca/cepae2.cfm?screen=Documents/office&Language=en</p>
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D. Decide if a field monitoring project is required

This step asks the question: “Does the potential level of environmental damage and the need for information justify the cost of a monitoring program and the safety risks associated with implementing it?” Any significant monitoring project will cost many tens of thousands or even millions of dollars. The ultimate use of the results of monitoring should justify the cost of the project³.

The factors to be considered are below.

- a) The value of the species populations and their vulnerability to the spill must be factored into the decision to monitor. Potential damage to all fish populations is important and is of interest scientifically. On the other hand, potential damage to commercially important or protected species always requires monitoring, if financial compensation is involved.
- b) The nature of the receiving environment and its capacity to dilute and dissipate the oil. Offshore marine waters have a large capacity for dilution and so can dilute the spill before significant damage is done. For that reason, spills far offshore may not require monitoring, unless they are large. On the other hand, marine spills in shallow nearshore areas have less capacity for dilution and a higher potential for impact and hence are more likely to require monitoring. River spills have the least capacity for dilution and the greatest potential need for monitoring.
- c) Spill size – Large spills that can potentially cause significant and widespread environmental contamination/ damage and may require monitoring, while small spills that pose limited risk may

³ Recognize that the party responsible for the spill will ultimately pay for the monitoring work.

not. Oil well blowout spills are continuous and can potentially be very large if they continue for an extended period and therefore generally require monitoring.

- d) Oil persistence – Marine spills of non-persistent oils may not require monitoring if the oil evaporates quickly. The more persistent the oil the greater the potential impact and need for monitoring. In rivers, on the other hand even non-persistent oils can be entrained before evaporating if there is sufficient mixing energy.

Hence, if the spill is relatively small (i.e., less than 1 m³) and not persistent, a field monitoring project might not be necessary. However, if the spill is large, persistent or threatens to injure exploited species or water supplies or poses a health or safety threat to the local human population, a monitoring program may be justified.

E. Coordinate with other monitoring programs operating for this incident

For most drilling-related spills and significant spills of other types, there will be several teams monitoring the behaviour and effects of the spill for a variety of purposes (e.g., response planning, fishery management). These teams may be generating information on the day-to-day behaviour, movements and spatial distribution of the spill that may be useful in designing a plan for monitoring effects on fish. In addition, other response teams will be a potential source of information on spill countermeasure activities (e.g., oil burning, dispersants, shoreline cleaning) that may influence the behaviour and movement of oil that may not be apparent from surveillance results. Communication among response organizations and monitoring teams is critical in deploying an effective and cost-effective monitoring operation.

In any sizeable spill monitoring, teams may include:

- a) The oil behaviour monitoring team which includes activities to measure the movements and persistence of oil and the spatial extent of oil on the water surface for purposes of managing the oil spill cleanup.
- b) The shoreline monitoring or Shoreline Cleanup and Assessment Technique (SCAT) team that monitors shoreline oiling for purposes of planning cleanup.
- c) The Canadian Food Inspection Agency team monitors spill-related contamination of fish habitat and fish tissues for purposes for managing fishery closures and other aspects of managing spill impact on the fishery.
- d) The wildlife monitoring team monitors the risks and impacts of the spill on birds, mammals and reptiles.

Activities of the teams may be coordinated through the Environment Canada's National Environmental Emergencies Center (website: <http://www.ec.gc.ca/ee-ue/default.asp?lang=En&n=8A6C8F31-1>, telephone: 514-283-2333 or 1-866-283-2333).

F. Conduct initial and subsequent spill area reconnaissance

It is important to make an early reconnaissance of the spill area in order to develop a first-hand understanding of:

- a) the nature of the spill
- b) the spatial scope of the spill and the fate of the spilled oil;

- c) the movement and persistence of the oil;
- d) the environmental compartments contaminated; and
- e) the acute environmental damage caused.

While many aspects of spill behaviour and contamination can be learned from the initial notification and situation reports, first-hand observations of the spill are critical to gaining an accurate appreciation of spill behaviour, scope and impact.

The objectives of the initial reconnaissance are to verify:

- a) The spill source and conditions, spill volume, properties and initial behaviour;
- b) Actual fate, movement and persistence of the spill as opposed to model outputs, and other observers' perceptions of these behaviour;
- c) Level and spatial scope of environmental contamination (i.e., water surface and shorelines)
- d) Evidence of potential environmental damage (e.g., wildlife) and actual damage (i.e., deceased and distressed wildlife and fish); and
- e) Spill response activity (e.g., containment and recovery, diversion booming, dispersants, burning, shoreline cleanup).

Objectives of subsequent reconnaissance sorties will vary as details of spill behaviour, movements and impacts are revealed. Reconnaissance should by aerial surveillance, but surface observation can also be helpful. Data are recorded manually using notes, sketches, photos and video. Appropriate forms have been developed for this purpose by various agencies. Surveillance activities should be planned as part of pre-spill preparations and should utilize available guides developed for this purpose by professional organizations (see Table 9).

Table 7: Published Spill Reconnaissance Guides for Monitoring Teams

Australian Maritime Safety Authority (AMSA). 2003a. Oil spill monitoring handbook. AMSA, Canberra
BC Ministry Environment. 2013. British Columbia Inland Oil Spill Response Plan. BC Ministry Environment.
International Tanker Owners Pollution Federation (ITOPF). No date. Aerial observation of marine oil spills. ITOPF Technical Information Paper #1.
International Tanker Owners Pollution Federation (ITOPF). No date. Recognition of oil on shorelines. ITOPF TIP
International Tanker Owners Pollution Federation (ITOPF). No date. Leadership, command & management of oil spills. TIP
International Tanker Owners Pollution Federation (ITOPF). No date. Effects of oil pollution on fisheries and mariculture. TIP
International Tanker Owners Pollution Federation (ITOPF). No date. Sampling and monitoring of marine oil spills. TIP
New Jersey Department of Environmental Protection. No date. Oil Spill Tool Kit For Citizens Monitoring Emergencies. NJ Dept Environmental Protection

G. Identify the monitoring objectives

As early as possible in the incident, identify the specific purpose and objectives of monitoring. As mentioned earlier, monitoring has been used for a variety of purposes in the past including for assessing

risks to of transmission of contaminants to humans, tracking contamination or damage to exploited fish populations, evaluating effectiveness of response, and supporting post-spill restoration efforts.

Regardless of the purpose, monitoring will involve determining some or all of the following:

- a) Presence or absence of contaminants or impact biomarkers in the environment or populations near the spill site (e.g., minor sublethal effects, majority sublethal effects, major trauma, mortality);
- b) The spatial scope of contamination or impacts;
- c) Linkage between observed impacts to spill; and
- d) The length of time needed for impacted populations to recover.

H. Identify the species to be monitored

Based on knowledge of the local species, identify several that are suitable subjects for monitoring. These may include species of interest in the area threatened by the spill, such as sentinel species or exploited species.

As discussed above, considerations include:

- a) Abundance and spatial distribution of species within and beyond the areas threatened by oil;
- b) Availability of nearby areas with populations of the same species that could serve as uncontaminated control areas for comparative purposes (i.e., similar habitat, same species, age distribution, conditions, physical conditions); and
- c) Availability of information concerning baseline levels of biomarkers in potential monitoring species prior to the spill (e.g., tissue levels of PAHs, EROD levels, information on spatial or seasonal variation in these).

In past spills, workers have favoured sedentary or less mobile species (e.g., bivalve molluscs, such as mussels) and species that are clearly in contact with sediments (e.g., flatfish) as monitoring species. However, when determined to be appropriate, more mobile species of species have been used for monitoring purposes.

In general, monitoring species should include at least the species that are exploited locally for commercial, subsistence and recreational purposes, and/or other abundant species recognizing the following:

- a) Species with broad distribution in the region – species that are broadly distributed , so that the same species will be available for sampling within impact areas and in adjacent unimpacted areas;
- b) Abundant species – species that are sufficiently abundant in impacted and control areas to allow sufficient numbers of individuals to be sampled for statistical analysis; and
- c) Species representative of specific habitats – species from specific habitats (e.g., benthic, pelagic) favouring species that are not highly mobile (i.e., sedentary or not highly mobile species such as bivalve molluscs or flatfish).

I. Identify the biomarkers to be used in monitoring

A broad diversity of conditions and effects are caused when fish, shellfish and marine mammals are exposed to hydrocarbons during a spill. These include mortality, a broad range of sublethal effects

(e.g., gross pathology, reversible stress responses) and accumulation of hydrocarbons in organism's tissues. These are referred to collectively as "biomarkers" of oil spill effects. The main interest in monitoring is detecting impacts that are significant at the population level (e.g., mortality, retarded growth, reduced reproductive potential) or sublethal effects (e.g., lesions, histopathology). However, these effects may not be readily evident in all spill situations. As a result, researchers have come to rely on more subtle indicators of hydrocarbon exposure that can be detected more readily and can be measured more economically (e.g. presence of PAHs or PAH metabolites or detoxifying enzymes in tissues) (See Table 10). Extensive research has been devoted to calibrating these biomarkers against concentrations of hydrocarbon. Additionally, work has been conducted to correlate the occurrence of subtle effects with more severe toxic effects in the population.

A number of biomarkers have been studied and used for monitoring environmental effects in fish. Van der Oost et al. (2003) has suggested a scheme for grouping these according to the level of severity of organismic response. The ranking system is as follows.

- a) Exposure monitoring – refers to measuring the concentrations of spill-related hydrocarbons in the abiotic environment to which biota are exposed.
- b) Bioaccumulation monitoring – refers to measuring the hydrocarbons and their metabolites accumulated into the organisms' tissues.
- c) Biological effect monitoring – refers to determining the early adverse alterations or responses in the organism to hydrocarbons that are partly or fully reversible (i.e., biomarkers). These include the biochemical and physiological responses used by the organism to protect itself from toxicants.
- d) Health monitoring – refers to examining the occurrence of irreversible disease, tissue damage or mortality in exposed organisms.

In the paragraphs below, biomarkers are organised using Van der Oost et al.'s (2003) system. In selecting biomarkers for a monitoring study it might be useful to select one or two markers from each group. In selecting biomarkers, recognise that certain other factors must be considered in selecting biomarkers for oil spill impact including:

- a) Favour those that are unlikely to be caused by non-spill related factors such as other forms of environmental contamination, other environmental stressors (e.g., heat) or periodic biological processes (e.g., reproductive cycle);
- b) Where possible, select biomarkers for which existing background in the population is low enough to allow effects of the current spill to be readily identified; and
- c) Where possible, select those that can be readily related to effects at the population level (e.g., growth, reproductive success).

Brief descriptions of common biomarkers and monitoring methods are provided below by category described in Van der Oost et al. (2003):

a) Exposure Monitoring

Exposure monitoring involves tracking the spatial, temporal and chemical changes in the distribution of contaminants in the abiotic environment, as the spill incident proceeds (e.g., oil slick, water, sediments, etc.). During a large oil spill, it will be essential to track the movements and persistence of oil slicks, contamination of shorelines, seabed sediments and the water-column in order to understand

the spatial extent of environmental exposure to oil. Some aspects of this monitoring (e.g., slick movement and shoreline oiling) may be undertaken by other members of the spill response team, as part of the cleanup efforts. The monitoring team should consider collecting some oil samples from the source, slicks and shorelines, as part of the effort to link environmental contamination to the spill. The monitoring team should undertake sampling of the water column and seabed to understand exposures in these compartments. Water and sediment samples are collected using standard oceanographic/limnological sampling methods. Standard methods for sample handling and preservation, as well as for analysis of PAHs in oil, environmental and biological samples are described by Kelly et al. (2000). Results of this monitoring will be essential for planning the collection of fish and shellfish.

New Developments in Exposure Monitoring

One new technique that has been used in exposure monitoring is the semi-permeable membrane device (SPMD). This device is used to sample lipophilic environmental contaminants like PAHs, in aqueous, sediment, and atmospheric media (e.g., Shigenaka and Henry 1995). SPMDs mimic biological systems to provide a measure of bioavailable pollutants in both fresh and salt water. Their passive transport mechanism (i.e., uptake) is similar to that of fish gills. In contrast to biota, SPMDs do not metabolize the sequestered compounds, are site-specific, are much easier to extract, and continues to monitor the environment even beyond levels that would normally cause mortality in biota. SPMDs and their use in monitoring are described in Alvarez (2010). A recent study considered the use of SPMDs in predicting PAH body burdens in biota (Forsberg et al. 2014).

b) Bioaccumulation monitoring

Bioaccumulation monitoring involves measuring contaminant levels in the tissues of monitoring species. Tissue contamination with spill-related hydrocarbons is the simplest and most unambiguous indicator of significant exposure of biota to spilled oil. Many compounds in oil are bioavailable and readily taken-up from water, food and sediments. These compounds can then appear quickly in tissues of exposed individuals. On the other hand, some of these compounds are also depurated quickly and therefore, may only be present in tissues for a brief period following exposure. The latter notwithstanding, measures of tissue contamination with PAH (and their metabolites) are some of the most commonly used markers for monitoring oil exposure during spills (Table 10). They are measured in virtually all monitoring studies to help linking injuries to the spill itself. Bioaccumulation of PAH in aquatic species has been reviewed by Meador et al. (1995). The use of tissue contamination in monitoring for spills has been reviewed by Law and Hellou (1999). Standard methods for analysing PAH in oil in environmental and biological samples are described by Kelly et al. (2000).

c) Biological Effects Monitoring

Biological effects monitoring involves measuring indicators of the early biochemical or physiological responses that organisms display when exposed to contaminants. In many cases these responses are partly or fully reversible.

Detoxification Enzymes

Most animals have some capacity to process contaminants such as PAHs to reduce or prevent damage to cells and biochemical functions. The first phase in the metabolism of absorbed hydrocarbon

contaminants is the production of enzymes to catalyze transformation reactions. The most important enzymes that catalyze detoxification processes are those using the cytochrome P-450 electron transfer system or mixed-function oxidase system (i.e., MFO enzymes). This family of enzymes includes aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-O-deethylase (EROD). MFO enzymes are present in many organs (e.g., gills, liver, intestines, and kidneys), but are usually most active in the liver. The possibility that biomarkers might be used to assess the health status of organisms and to obtain early-warning signals of environmental risks was first suggested by Payne et al. (1987).

Measures of MFO activity can be used to demonstrate exposure and response to contaminants (Morales-Caselles et al. 2006). These biomarkers are not necessarily indicators of actual adverse effects in the organism as they are produced by the organism to enable metabolism, binding, and/or excreting of contaminants and thus, in many cases, for preventing adverse effects. In some cases, however, the presence of these biomarkers in the organism can also be indicative of adverse effects. Another difficulty in the interpretation of enzyme activity levels is that some of these enzymes may also be produced in response to changes in temperature, reproductive state, or capture stress. In spite of these difficulties, under conditions where exposure to contaminants cannot be demonstrated with other measures, MFO enzyme activity is a useful measure of contaminant exposure (Kirby et al., 1999). For that reason, the measure of MFO enzymes is one of the most commonly used biomarkers in oil spill monitoring (Table 10).

A method for measuring EROD in fish tissues is provided in Appendix 2.

Binding Proteins (GSH, GST)

The production of MFO enzymes increases the organism's detoxification capacity for the chemicals to which it is exposed. The second phase in the transformation of contaminants (i.e., conjugation reactions) depends on the availability of conjugating substances produced within the organism (e.g., concentration of binding or scavenging proteins). For organic contaminants, one of the more important conjugating compounds is the peptide, glutathione (GSH), which sequesters oxygenated metabolites away from sensitive cellular sites. The activity of this and other conjugating substances may be increased or induced by exposure to various classes of organic compounds. Enzymes that catalyze reactions with GSH have been studied as biomarkers. These glutathione transferases (GST) also bind to contaminant metabolites and appear to be elevated in fish, crabs, and mussels from sites contaminated with PAHs (Stegeman et al., 1992). Moriera et al. (2004) in a monitoring operation for the "Coral Bulker" fuel oil spill off the coast of Portugal demonstrated that elevated GST levels in mussels correlated well with levels of environmental hydrocarbon contamination. On the other hand, Van der Roost et al. (2003) argued that hepatic total GST activity in fish may not be feasible as a biomarker for monitoring, since increased GST activities after PAH exposure may only be observed in some species.

A method for analysing for GST is provided in Appendix 3.

Contaminant Metabolites in Bile

Several aquatic species can metabolise PAHs forming metabolites that are generally more water soluble than their parent compounds. PAH metabolites formed by the MFO system can be hydrophilic products such as phenols and quinones. The formation of these more water soluble products increases the rate of their excretion from the organism. These metabolites are sometimes transferred directly in bile as

unconjugated polar metabolites. However, most PAH metabolites will be conjugated by phase II enzymes (including glucuronidation) before excretion. Since PAHs are readily depurated and metabolized in aquatic species, several authors argue that the presence of metabolites in liver or bile may be more useful biomarkers for exposure to oil than the parent compounds. They have been used successfully in monitoring in many spills including the Apex Barge spill (McDonald et al. 1991) and the Erika spill (Budzinski et al. 2004)

According to Budzinski et al. (2004) PAH metabolite concentrations in bile are usually determined semi-quantitatively as global fluorescent aromatic compound equivalents (FACs) in fish (Beyer et al. 1996; Aas et al. 2000). However, they can be measured using several analytical methods, including:

- a) Simple fluorescence assays (fixed fluorescence detection or synchronous fluorescence spectrometry); high-performance liquid chromatography with fluorescence detection (HPLC-F);
- b) Gas chromatography-mass spectrometry (GC-MS) after deconjugation, extraction and derivatization of the bile sample;
- c) Advanced liquid chromatography-tandem mass spectrometry (LC-MS/MS) and
- d) Gas chromatography-tandem mass spectrometry (GC-MS/MS) methods.

These methods have been described and reviewed by Beyer et al. (2010). A detailed description of a method for analysing for metabolites of glucuronide metabolites of alkyl-phenanthrenes is provided in Turcotte (2008). A detailed description of a method for analysing for metabolites of pyrene in fish impacted by the Erika spill is provided in Budzinski et al. (2004). A method for measuring PAH metabolites in bile is included in Appendix 4.

PAH-DNA Adducts

In some cases, PAHs metabolism in biota leads to the production of active metabolites that can interact with DNA and cause the formation of DNA adducts. The formation of PAH-DNA adducts is a critical part of the process by which PAH exposure produces hepatic carcinogenicity in fish. The carcinogenicity of PAH varies depending on compounds involved and also by fish species. For example, the compounds benzantracene, chrysene, benzo(a)pyrene and benzo(k)fluoranthene have been known to cause tumours in fish (de Maagd and Vethaak, 1998). PAH-DNA adducts have become recognized as a reliable and useful biomarker for both PAH exposure and biological effects and have been used in monitoring in spills including the Sea Empress spill (Harvey et al. 1999) and Erika spill (Amat et al., 2006). Methods for analysing for PAH-DNA adducts are described in detail in a number of publications including Amat et al. (2006) and Kirby et al. (2000).

d) Health Monitoring

Health monitoring refers to assessing the occurrence of injuries that are significant at the level of the individual or population. These include tissue damage, retarded growth or impaired reproduction.

Histopathology

The histopathological effects of oil on fish have been recognized since at least the Amoco Cadiz oil spill in the late 1970s (Haensly et al. 1979). These biomarkers have been widely used in environmental monitoring of fish because:

- a) Specific target organs (e.g., gills, kidneys, liver and gonads) that are known to be sensitive to oil exposure are examined for evidence of injury;
- b) These organs are responsible for vital life functions that may be disrupted by organ damage; and
- c) They are involved in the accumulation/biotransformation of PAHs within the body of the fish (e.g., Au 2004).

In finfish, the liver is the primary organ for biotransformation of organic xenobiotics (Hinton et al. 2001) and is therefore commonly used in monitoring studies. Other tissues, particularly gills can also be used. Pathological effects vary with the tissue studied and the level of exposure. Hinton et al. (1998) reported finding hepatic lipidosis, megalocytosis and hepatocellular necrosis in livers of finfish from heavily oiled environments during the Exxon Valdez spill. Other histopathology studies during spills include Amoco Cadiz (Haensly et al. 1979); and Prestige (Marigomez et al. 2006).

Methods used for collecting, preserving, sectioning and staining tissue for histopathological study will vary with the tissue and lesions involved. An example method used by Marigómez, et al. (2006) for preparing fish liver tissue is provided in Appendix 6.

Gross Pathology

When cellular injury occurs and major portions of tissues or organs become involved, it is sometimes possible to observe the damage by external examination of skin, eyes and fins of organisms. Fin erosion is one of the most easily detected external abnormalities in fish and was reported in flatfish in heavily oil contaminated bays during the Amoco Cadiz oil spill (Haensly et al. 1982). Epidermal tumors and skeletal abnormalities are also commonly observed in contaminant studies. There have been numerous accounts in the media of deformities and lesions in fish and shellfish following the Gulf of Mexico spill (2010) but to date there have been little information in the scientific literature to support a link to the 2010 oil spill. The value of these gross injuries in monitoring is that a) they indicate that significant sublethal effects have occurred and b) they are clearly visible to the unaided eye.

Immune System Responses

Payne and Fancey (1989) were among the first to demonstrate immunosuppression by PAH exposure in finfish. Auffret et al. (2004) and others have done so using invertebrates. The latter used immunosuppression as a monitoring tool during a spill (i.e., Erika 1999). However, according to Barron (2012) immunity suppression is not used with regularity as a monitoring tool in oil spills. Barron argued strongly that immunosuppression might be an important consequence of spills and should be included in monitoring, particularly for large spills like the recent Gulf of Mexico spill in 2010. The subject of immunosuppression in fish and shellfish has been reviewed by several authors (e.g., Reynaud and Deschaux 2006; O'Halloran et al. 1998). A variety of measures of immune system response have been used as indicators of contamination, including blood cell counts, kidney macrophage function (known to be sensitive to PAHs in fish), and specific antibody counts. Some methods for conducting immunosuppression testing in fish are described in Skouras et al. (2003).

The fact that immunosuppression has not been commonly used a monitoring tool in spills, suggests that it might not be the first or only choice for monitoring for a major spill in Canada in the near future. However, the linkage between PAH exposure and immunosuppression in fish and mammals is clear and the potential impact of widespread spill-induced immunosuppression in a population during a

spill cannot be ignored. For that reason it would be useful to develop an understanding of immunosuppression in Canadian species and the potential long term consequences for individuals and populations (e.g., salmon, snow crab, Arctic cod).

Growth

The impact of oil exposure on growth in fish and shellfish was observed as early as the Amoco Cadiz spill in 1979. Following that spill, Conan and Friha (1979) showed that sole and plaice from oiled bays on the French coast grew significantly more slowly than in clean bays. Subsequently Vignier et al. (1992) and Moles et al. (1998) demonstrated experimentally that prolonged exposure (10-90 days) to oil in water or sediments, respectively, could cause significant reductions in growth in salmon and flatfish. Vignier et al. (1992) also demonstrated that the reduction in growth was caused by reduced efficiency of food conversion rather than reduced feeding rate. Peteiro et al. (2006) demonstrated a significant effect of oil contamination on growth in farmed mussels after the Prestige spill. However, fish growth does not appear to have been used as frequently in spill monitoring as other biomarkers.

Disruption of Reproduction

Reproduction in fish is a complex process that is governed by an intricate system of hormonal, nutritional and external controllers. Chemicals like PAHs can disrupt reproduction in a number of ways and has been identified fish populations in past spills.

These effects include the following:

- a) Reduced size of gonads/gonad-somatic ratio in exposed adults (e.g., Amoco Cadiz , Moissec (1981));
- b) Reduced fecundity in exposed adults (e.g., Amoco Cadiz, Leglise and Raguenes (year));
- c) Reduced viability of eggs and larvae due to exposure of adults or young to oil (e.g., Exxon Valdez, Kocan et al. (1996)); and
- d) Change in population age structure (Amoco Cadiz, Desaunay (1981)).

It should be noted that cases where reproductive impairment has been identified involved large spills of persistent oils. Measurements of actual reproductive parameters may be costly and time-consuming and linkages of apparent effects to the spill may be challenging. However, these parameters are unambiguous indicators of effects at the population-level and would be useful to consider when possible.

Table 8: Examples of Sublethal Biomarkers in Fish/Shellfish Used in Monitoring Oil Spills

Biomarkers	Biological Significance	Timescale for measuring response	Tissue collected	Test method and reference	Examples of Application (<i>organism - spill name (reference)</i>)	
					Mollusks	Fish
PAH compounds (parent compounds)	Indicates exposure to PAHs. Measure of parent compounds can underestimate exposure level.	Hours, days, months	muscle, liver	gas chromatographic methods (reviewed in Poster et al. 2006), others	Mussels - EVOS (Boehm et al. 2004); Mytilus - Don Pedro spill (Sureda et al. 2011); Razor clam - Prestige (Vinas et al. 2009); Mytilus - EVOS (Carls et al. 2001)	Fish - Hebei Spirit (Jung et al. 2011); Fish - Madagascar oil spill (Rumney et al. 2011); Pacific Herring - EVOS (Carls et al. 2001); Fish/Shellfish- Braer (Kingston 1999)
PAH metabolites (products of PAH degradation via metabolic pathways)	Indicates exposure to PAHs and includes ongoing and recent exposure to compounds derived from parent compounds.	Hours, days, months	bile	biliary fluorescent aromatic compounds, others (several methods reviewed in Beyer et al. 2010)	generally not used (low biotransformation capability, Beyer et al. 2010)	Rockfish - EVOS (Page et al. 2004); Rockfish - EVOS (Marty et al. 2003); Fish - Hebei Spirit (Jung et al. 2011).
Detoxication enzymes	Indicates induction of detoxication mechanisms. Involves cytochrome P450 enzyme system.	Days to months	liver, gills, others	EROD activity (ref), CYP1A protein (ref)	Mytilus - Don Pedro spill (Sureda et al. 2011); Mytilus - Prestige (Marigomez et al. 2006).	Benthic fish spp-EVOS (Huggett et al. 2003); Rockfish - EVOS (Page et al. 2004); Fish - Hebei Spirit (Jung et al. 2011).
ACHE inhibition	Indicator physiological stress	Hours, days, months	gills	measure of acetylcholinesterase (ACHE) activity (ref)	Mytilus sp. - Erika (Bocquené et al. 2004)	generally not used (was applied in monitoring by Jung et al. 2011)

Table 8: Examples of Sublethal Biomarkers in Fish/Shellfish Used in Monitoring Oil Spills

Biomarkers	Biological Significance	Timescale for measuring response	Tissue collected	Test method and reference	Examples of Application (<i>organism - spill name (reference)</i>)	
					Mollusks	Fish
Oxidation reactions and responses	Indicates oxidative stress as a result of PAH detoxication and others.	Hours, days, months	digestive gland (mollusks only), blood, liver, gills	Examples include: lipid peroxidation, glutathione peroxidase, superoxide dismutase (Livingstone et al. 1993)	Mytilus - Aegean Sea spill (Solé et al. 1996); Mytilus sp. - Erika (Bocquené et al. 2004).	coho salmon - EVOS (Roberts et al. 1996)
Genetic disorders	Indicates genotoxic damage, i.e., damage to genetic material.	hours, days, months	gills, blood, liver, kidney, digestive glands	Examples include: alkaline comet assay (Wilson et al. 1998), micronucleus test (Venier et al. 1997), DNA adducts (Gupta et al. 1982), chromosomal aberrations (Al-Sabti and Kurelec 1985), alkaline filter elution (Goumenou et al. 2004)	Mytilus sp. - Prestige spill (Pérez-Cadahia et al. 2004); Mytilus sp. - Erika (Bocquené et al. 2004); mussels and oysters - Haven spill (Bolognesi et al. 2006)	Fishes - Sea Empress spill (Harvey et al. 1999); benthic fish - Haven spill (Bolognesi et al. 2006)
Histopathology	Indicates damage to tissues and disease. Useful to detect early signs of stress prior to advanced abnormalities.	Months, years	Gill, kidney, liver, gonads, intestines	Examples include: lesions and inclusions, neoplastic formations, ovotestis (intersex) (Stentiford et al. 2003)	Mytilus - Prestige spill (Garmendia et al. 2011); Mytilus - Prestige spill (Cajarville et al. 2006); Mytilus - Prestige (Margomez et al. 2006).	Bream - Po R. Spill 2010 (Giari, et al. 2012); Herring - Exxon Valdez (Marty et al. 1999) Rockfish - EVOS (Marty et al. 2003); Mytilus - Prestige (Marigomez et al. 2006).
Gross pathology	Indicates physiological stress, disease, suppressed immune system, others.	Months, years	Eyes, skin, fins, internal organs (large tumors)	external and internal examination (e.g., fin erosion or rot, tumors, other abnormalities)		Red Snapper - Macondo - Arias et al. 2013; fishes - Amoco Cadiz spill (Conan et al. 1982)

Table 8: Examples of Sublethal Biomarkers in Fish/Shellfish Used in Monitoring Oil Spills

Biomarkers	Biological Significance	Timescale for measuring response	Tissue collected	Test method and reference	Examples of Application (<i>organism - spill name (reference)</i>)	
					Mollusks	Fish
Growth	Correlated to physiological stress, others.	Months, years	n/a	short-term measures using caged animals, long-term measures of individuals from wild populations	Mytilus sp. - Prestige spill (Peteiro et al. 2006)	pink salmon fry - EVOS (Rice et al. 2001)
Reproduction-related	Correlated to physiological stress, endocrine disruption, others.	Months, years	blood, gonads	Examples include: gonad histology (see Histopathology), gonadosomatic index, sex ratios, measure of reproductive hormones (e.g., estradiol-17B, Sower and Schreck 1982; gonadotrophin-I, Swanson et al. 1989)	Mytilus - Prestige spill (Ortiz-Zarragoitia et al. 2011)	fish spp. - EVOS (Sol et al. 2000)

J. Identify the Monitoring Strategy

If monitoring has been decided to proceed, then the following list of priorities might be useful for selecting biomarkers and sampling strategies:

- a) In all spills, document the spatial and temporal extent of hydrocarbon contamination in the abiotic environment. This identifies the spatial extent of exposures and the locations of most severe exposures. Use the daily reports on distributions of oil slicks and contaminated shorelines to develop a sampling plan for contamination of sediments and the water-column.
- b) In large spills and those with potential for persistent contamination of seabed or shorelines over a large area, determine whether there is significant tissue contamination with PAH and PAH metabolites within or near the contaminated area.
- c) If tissue contamination is present, then test for sublethal responses and tissue damage in areas of where tissue contamination is greatest.
- d) If sublethal responses and tissue injuries are present (e.g., histopathologies), then: a) determine the spatial extent of biomarkers in the population; and b) link the observed impacts to the spill.
- e) Decide which biomarkers are to be tracked for recovery and initiate that practice.
- f) Decide whether health and reproduction biomarkers are to be tested and which ones.

Historically, monitoring teams have used one of three general strategies for scientifically detecting spill-related changes in fish populations near spills. Use any one or combinations of these, as appropriate:

- a) Comparing post-spill biomarker levels in organisms in contaminated areas to pre-spill levels in the same locations to identify significant changes. This strategy is possible in spills from petroleum installations, where data on pre-spill conditions are generally gathered as part of routine environmental effects monitoring activities (e.g., Husky Energy 2010).
- b) Comparing levels of biomarkers near the spill with those of uncontaminated areas distant from the spill.
- c) Tracking the post-spill recovery of contaminated/impacted areas/populations back to pre-spill conditions over time after the spill has been stopped and cleaned up. Numerous initiatives of this kind were undertaken after the Exxon Valdez oil spill in Alaska in 1989 (see http://www.evostc.state.ak.us/index.cfm?FA=searchResults.projectInfo&Project_ID=2239).

K. Identify the Spatial Scope of Monitoring Activity and Identify Reference Sites.

One of the critical objectives of monitoring is to determine the amount of injury inflicted by the spill. One of the most useful measures of this is the spatial extent of the damage. This information is useful in the short term in managing the secondary impacts of the spill on the local fishing community (Kingston 1999). For river spills, this means determining how far down river spill effects have occurred. In marine environments and lakes it means determining the spatial extent in two dimensions. As explained above, during spill incidents, the size of the area impacted grows during the early phases of the spill and shrinks in the later recovery phases. While the behaviour of the surface oiling may be tracked visually, contaminant behaviour in bed sediments may be determined only through monitoring results. To determine the largest spatial extent of spill impact, it will be important to track two things:

- a) The extent of contamination (slicks, water column, sediments, oiled shorelines); and

- b) The spatial extent of the effects within the population (biomarkers).

Determining some aspects of the area of environmental contamination may be outside the area of responsibility of the present manual. Other members of the response team will be addressing this using computer oil fate modeling, visual observations, remote sensing and environmental sampling. The results of their work will be critical to planning for EEM. For purposes of EEM develop a series of sample sites designed to delimit approximately the edges of the area of contamination. Recognize that the distribution of habitat types in the area being sampled may be patchy and the distribution of hydrocarbons within these areas may be patchy as well. Depending on the spill conditions and the size of the area involved a sampling grid or transects radiating from the main spill site may be useful initially, transitioning to a series of stratified sampling sites as the distribution of oil contamination becomes evident in the later stages of the spill. Guidance in selecting sampling areas and in determining sample numbers is provided in (ITOPF No Date, page 4).

Reference Sites

At this time identify several potential “control” or “reference (no contamination)” sites. These must involve sites outside the potential area of impact that contain the same species that are being sampled near the spill site and similar habitat (e.g., substrate), but must be free of spill related contamination, have similar physical habitat (e.g., temperature, water depth) and be free of other environmental stressors that might have complicating influence on biomarker activity.

L. Establish criteria for terminating monitoring

At this point establish criteria and conditions for terminating monitoring (e.g., when certain biomarker endpoints have returned to background levels). Criteria will almost certainly evolve during the course of the project but it is useful to set explicit termination criteria from the outset.

M. Prepare a formal monitoring plan

Prepare a formal monitoring plan that addresses the monitoring objectives and subjects/activities described above. An example table of contents is provided in Table 11. Guidance in preparing a plan is available in ITOPF Technical Document #14. Use appropriate, accepted analytical methods for parameters being tested (see appendices). In preparing and implementing the monitoring plan, several key items will be useful in ensuring the effectiveness of the plan and its execution. These include:

- a) A suitable map or chart to visually track the oil fate, habitats at risk, distributions of monitoring species, sampling sites and results;
- b) An organization chart identifying all internal personnel and groups (and contact information) executing the various phases of the plan, as well as external links to other groups involved in the response (e.g., logistics); and
- c) Flowcharts describing the handling of each type of sample being gathered / analysed, the information generated and how it is to be communicated to end users on a routine basis.

Background information regarding most sections of the plan have been provided above or in the appendices that follow with the exception of the subjects of selection of sample sites, sample numbers and frequency.

Table 9: Example Table of Contents for Monitoring Plan

Monitoring Project Overview
Summary Spill Conditions, Movement and Fate
Monitoring Objectives
Monitoring Strategy
Preliminary Reconnaissance
Species and Biomarkers
Location of Sampling
Timing of Sampling
Organization
Types of Samples (Oil, Environmental, Biota)
Sampling Methods
Sample Handling Methods and Labeling
Analytical Methods
Quality Assurance and Control
Interpretation and Communication
Termination
Budget

Field Sampling Plan

Field surveys can be useful for rapidly collecting geographically-referenced information on the location and extent of the oil. Surveys can also be useful for monitoring qualitatively the effectiveness of clean-up operations or the progress of natural recovery. The location and number of sites that should be included in field surveys or sampling stations will depend largely on the fate and movement of the oil and the variability of both the environment and oil movement within it. Care should always be taken to ensure that the sites chosen are representative of the habitats being monitored. However, most oil spill scenarios do not require the use of sophisticated statistical procedures to determine the number of sites to be surveyed or the number of samples to be collected. In reality, compromises and pragmatism are often needed in order to satisfy both the demands of statistical reliability and the practicality of accounting for the full spatial and temporal variation of complex ecosystems within the available time frame and financial constraints. Furthermore, there are few universal rules regarding the optimum location and number of sampling stations for post-spill monitoring studies. Instead, this will depend on the objectives of the monitoring programme and on a number of case-specific variables such as the physical characteristics of the area affected, and the nature and location of fish populations to be monitored sensitive resources; and physical conditions that might constrain sampling (e.g. access or weather).

In practice, sampling stations should be selected to reflect the distribution of oil and natural physical and biological environmental gradients. Entirely random approaches to sampling are possible, but they are rare when sampling solely for contaminants in a monitoring programme. Although a random approach would enable greater use of statistical inference in the reporting of results, a significantly large number of samples would need to be analysed at much greater cost for little improvement in the data obtained. Instead, in complex cases, a useful compromise can be achieved by making certain elements of the study random, for example, using stratified random sampling, or implementing more sophisticated phased (i.e., cluster) or composite sampling. Setting up an appropriate probability-based sampling design in such cases may call for the services of an environmental statistician.

Timing of the monitoring programme

The timing of sampling is important because during oil spills, evidence of environmental contamination and biological effects change with time; and do so rapidly during the early phases of the spill. Timing of sampling is important in two respects. First, the sooner sampling is started, the sooner short-lived (i.e., ephemeral) effects can be detected and the changing extent of contamination recorded. In later phases of the spill, changes take place more slowly and sampling frequency can be reduced.

N. Develop Monitoring Support Functions

Any spill response generates thousands of oil and environmental samples for a variety of purposes. Develop and apply a suitable system for labeling and tracking (i.e., chain of custody) samples and for assuring their proper handling and analysis.

Labeling

Labeling should include location and date, name of worker taking the sample, type of sample, purpose and type of handling required.

Chain of Custody

Chain of custody is a legal term referring to the ability to guarantee the identity and integrity of the specimen from collection through to reporting of the test results. It is a process used to maintain and document the chronological history of the specimen. Documents should include a unique identifier (i.e., specimen number) by which the sample can be identified, the name of the individual collecting the specimen, each person or entity subsequently having custody of it and its location, the date the specimen was collected or transferred, and a brief description of the specimen. Containers in which samples are transported and stored should be sealed with custody seals so that they cannot be opened without breaking the seal. A secure chain of custody, together with the use of robust, validated and quality controlled analytical techniques to confirm the identity and establish the concentrations of contaminants present in a specimen, leads to the production of valid and legally defensible data. Examples of chain-of-custody forms for registering changes of stewardship of samples are given as appendices in IMO (1998) and Yender et al. (2002).

Quality Assurance and Control

To maintain high quality of sampling and analysis, every monitoring plan should incorporate two key elements:

- a) quality assurance (QA) to ensure that processes and procedures are in place to check that the aspects of the monitoring plan, such as sampling and analysis, are being carried out in the correct manner (an audit of the process); and
- b) quality control (QC) to ensure that the monitoring plan delivers the planned objectives (a check of the product).

Samples may be divided in a number of ways for quality control purposes and this is decided prior to sample collection. Each fully-homogenised sample is divided after being drawn or taken, giving two or more parties the opportunity to undertake independent analyses. The same device and procedures are used at the same location to take two or more samples which should be identical. Such samples are used to test sample variance and their identity may not always be made known to the laboratory. In the laboratory, split samples given to the same laboratory for analysis yet described as being two different samples can be used to check the precision of laboratory analysis

O. Implement Plan

As early as possible in the development of the above plan, begin to assemble and deploy the personnel, equipment and logistics resources needed to execute the plan. Because of the need to collect some information as early as possible in the spill, identify the resources needed for implementing the response (e.g., human resources, finance).

P. Communicate monitoring results to end users

One of the most critical steps in spill impact monitoring (and the one most often neglected) is communicating the results to critical end users. The primary objective of monitoring is to inform:

- a) stakeholders concerning the impact of the spill on resources of interest; and
- b) responders concerning damage, effectiveness of response measures and the need for restoration.

It is critically important that the results of monitoring be communicated effectively to these groups, so that they can take appropriate action. At an early stage in developing your plan, clearly identify the end users for your monitoring results and identify precisely the information needed and why it is needed. Provide the monitoring results in an effective format for that purpose (graphs, tables, maps, etc.)

Q. Reassess plan periodically

Reassess the objectives and details of the plan as the spill incident proceeds. Spill conditions and countermeasures may change altering the oil exposure conditions. Preliminary monitoring results will provide information on oil fate, species sensitivity and risks. Be prepared to adjust the plan as spill circumstances change.

R. Terminate monitoring

Terminate monitoring when criteria established in Step L are satisfied.

5. Summary and Recommendations for Research and Planning

Activities of oil and gas industry pose a risk of oil spills into Canada's marine and freshwaters. These in turn pose risks to environmental and human health. During any spill, our response is guided, in part, by monitoring activities that are mounted during the spill, as well as by studies of previous spills. This document contains a brief overview of the types of oil spill scenarios potentially encountered in Canada in the near future, some background on spill-related monitoring and a brief guide to developing a an oil spill monitoring plan for use on spills in Canadian waters.

This report provides the following information. Section 2 provides information concerning the injuries caused to finfish, shellfish and mammals by oil spills into water, major reviews of the subject and past monitoring efforts during oil spills. Section 3 describes the types of spill scenarios that could potentially occur in the coming years, given the existing industrial setting in Canada. It also highlights new subjects that may not have been encountered by spill monitoring teams in the past. Section 4 contains a 14-step guide to implementing a monitoring plan for a major spill, addressing steps in planning and decision-making. The present section provides recommendations for specific areas of research and development that can improve our ability to mount monitoring efforts efficiently for spills in Canada. These deficiencies are as follows.

- 1) For potential Canadian biomonitoring species (cod, arctic cod, snow crab, salmon, flatfish, mussels, oysters) prepare an annotated bibliography of studies on effects and relationship among exposure, bioaccumulation of and effects of PAH and effects (e.g., biomarkers).
- 2) For representative species used for monitoring, determine the exposure-response-recovery relationships for biomarker activity and occurrence of histopathological changes.
- 3) Identify and acquire an off-the-shelf model for estimating fate, behaviour and persistence of spills into rivers or develop one.
- 4) Regarding the potential for spills in the Canadian Arctic:
 - a. Consolidate the rapidly expanding knowledge about the aspects of the ecology of arctic species relevant to monitoring (spatial distribution, populations, habitat use, seasonality); and
 - b. Prepare a review of research into effects of oil spills on Arctic marine and anadromous fish species (e.g., Arctic and polar cod, Arctic herring, Arctic char, Arctic cisco).
- 5) Develop a decision tree to help decide whether or not a field monitoring program is required during a spill incident.
- 6) Prepare an up-to-date directory for all of the regional environmental emergency coordination centres in Canada, including the names and contact information for the senior personnel.
- 7) Immunosuppression has been used in monitoring for oil spills in the past, but perhaps not as frequently as in other species. The linkage between PAH exposure and immunosuppression in fish and mammals is clear and the potential consequences for widespread immunosuppression in a spill exposed population cannot be ignored. For that reason, it would be useful to develop an understanding of the sensitivity of Canadian species to PAH-induced immunosuppression and the potential long-term consequences of it in Canadian fish populations during spills.

6. References

- Anonymous. 2013. The Use of surface and Subsea Dispersants During the BP Deepwater Horizon Oil Spill. Draft Report - National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling. 6 October 2010. Retrieved 17 February 2013.
- Arias CR1, Koenders K, Larsen AM. Predominant bacteria associated with red snapper from the Northern Gulf of Mexico. *J Aquat Anim Health*. 2013 Dec;25(4):281-9. doi: 10.1080/08997659.2013.847872.
- Alvarez, D.A., 2010, Guidelines for the use of the semipermeable membrane device (SPMD) and the polar organic chemical integrative sampler (POCIS) in environmental monitoring studies: U.S. Geological Survey, Techniques and Methods 1–D4, 28 p.
- AMSA. 2003. Oil Spill Monitoring Handbook. Prepared by Wardrop Consulting and the awthron Institute for the Australian Maritime Safety Authority (AMSA) and the Marine Safety Authority of New Zealand (MSA). Published by AMSA, Canberra. ISBN0 642 7099
- Anderson, C. 2001. Persistent Vs Non-Persistent Oils: What You Need to Know. Beacon, July 2001
- Anderson, A-M. 2005. Wabamun Lake Oil Spill August 2005: Data Report for Water and Sediment Quality in the Pelagic Area of the Lake (August 4-5 to September 15, 2005). Alberta Environment, April 2 Anderson, J.W., J. M. Neff, B. A. Cox, H. E. Tatem, G. M. Hightower Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish *Marine Biology* (1974):
- Amat, A., T. Burgeot, M. Castegnaro, A. Pfohl-Leszkowicz. 2006. DNA adducts in fish following an oil spill exposure. *Environ Chem Lett* (2006) 4: 93–99.
- Arias CR, Koenders K, Larsen AM. 2013. Predominant bacteria associated with red snapper from the Northern Gulf of Mexico. *J Aquat Anim Health*. 2013 Dec;25(4):281-9. doi: 10.1080/08997659.2013.847872.
- Au D. W. T. (2004). The application of histocytopathological biomarkers in marine pollution monitoring: A review. *Mar Pollut Bull* 48, 817–34.
- Barron, M.G. 2012. Ecological impacts of the Deepwater Horizon oil spill: implications for immunotoxicity. *Toxicol Pathol*. 2012;40(2):315-20.
- Beyer J1, Jonsson G, Porte C, Krahn MM, Ariese F. Analytical methods for determining metabolites of polycyclic aromatic hydrocarbon (PAH) pollutants in fish bile: A review. *Environ Toxicol Pharmacol*. 2010 Nov;30(3):224-44. doi: 10.1016/j.etap.2010.08.004. Epub 2010 Sep 18.
- Blenkinsopp, S., G. Sergy, K. Doe, G. Wohlgeschaffen, K. Li, and M. Fingas. 1997. Evaluation of the toxicity of the weathered crude oil used at the Newfoundland Offshore Burn Experiment (NOBE) and the resultant burn residue. *Proc. Twentieth Arctic and Marine Oilspill Program Technical Seminar*, Environment Canada, Ottawa, Ontario, pp. 677-684. Bocquene, G. et al. 2004. Biological effects of the "Erika" oil spill on the common mussel (*Mytilus edulis*). *Aquatic Living Resources*, 17(3), 309-316.
- Boehm PD1, Page DS, Brown JS, Neff JM, Burns WA. Polycyclic aromatic hydrocarbon levels in mussels from Prince William Sound, Alaska, USA, document the return to baseline conditions. *Environ Toxicol Chem*. 2004 Dec;23(12):2916-29.
- Born AF, Espinoza E, Murillo JC, Nicolaides F, Edgar GJ. 2003. Effects of the Jessica oil spill on artisanal fisheries in the Galápagos. *Mar Pollut Bull*. 47(7-8):319-24.

- Budzinski, H., O. Mazéas, J. Tronczynski, Y. Désaunay, G. Bocquené and G. Claireaux. 2004. Link between exposure of fish (*Solea solea*) to PAHs and metabolites: Application to the "Erika" oil spill. *Aquat. Living Resour.* 17, 329–334 (2004)
- Buist, I. et al. 1997. Laboratory Studies of the Properties of In-situ Burning. 1997 International Oil Spill Conference, 1997.
- Barron, M.G. 2012. Ecological impacts of the Deepwater Horizon oil spill: implications for immunotoxicity. *Toxicol Pathol.* 2012;40(2):315-20.
- Beyer, J., G. Jonsson, C. Porte, M. Krahn, Freek Ariese. 2010. Analytical methods for determining metabolites of polycyclic aromatic hydrocarbon (PAH) pollutants in fish bile: A review *Environmental Toxicology and Pharmacology* 30 (3):224–244
- Bocquene, G. et al. 2004. Biological effects of the "Erika" oil spill on the common mussel (*Mytilus edulis*). *Aquatic Living Resources*, 17(3), 309-316.
- Boehm PD1, Page DS, Brown JS, Neff JM, Burns WA. Polycyclic aromatic hydrocarbon levels in mussels from Prince William Sound, Alaska, USA, document the return to baseline conditions. *Environ Toxicol Chem.* 2004 Dec;23(12):2916-29.
- Hélène Budzinski1,a, Olivier Mazéas1,2, Jacek Tronczynski2, Yves Désaunay3, Gilles Bocquené2 and Guy Claireaux4 Link between exposure of fish (*Solea solea*) to PAHs and metabolites: Application to the "Erika" oil spill. *Aquat. Living Resour.* 17, 329–334 (2004)
- Christensen JH1, Tomasi G. Practical aspects of chemometrics for oil spill fingerprinting. *J Chromatogr A.* 2007 Oct 26;1169(1-2):1-22. Epub 2007 Sep 6.
- Cajaraville MP1, Garmendia L, Orbea A, Werding R, Gómez-Mendikute A, Izagirre U, Soto M, Marigómez I. Signs of recovery of mussels health two years after the Prestige oil spill. *Mar Environ Res.* 2006 Jul;62 Suppl:S337-41. Epub 2006 Apr 18.
- Christensen JH1, Tomasi G. Practical aspects of chemometrics for oil spill fingerprinting. *J Chromatogr A.* 2007 Oct 26;1169(1-2):1-22. Epub 2007 Sep 6.
- Da Silva Rocha AJ1, Gomes V, Rocha Passos MJ, Hasue FM, Alves Santos TC, Bicego MC, Taniguchi S, Van Ngan P. EROD activity and genotoxicity in the seabob shrimp *Xiphopenaeus kroyeri* exposed to benzo[a]pyrene (BaP) concentrations. *Environ Toxicol Pharmacol.* 2012 Nov;34(3):995-1003. doi: 10.1016/j.etap.2012.07.006. Epub 2012 Jul 31.
- Eide M, Karlsen OA, Kryvi H, Olsvik PA, Goksøyr A 2013. Precision-cut liver slices of Atlantic cod (*Gadus morhua*): An in vitro system for studying the effects of environmental contaminants. *Aquat Toxicol.* 2013 Nov 2. pii: S0166-445X(13)00297-X. doi: 10.1016/j.aquatox.2013.10.027.
- Cajaraville MP1, Garmendia L, Orbea A, Werding R, Gómez-Mendikute A, Izagirre U, Soto M, Marigómez I. Signs of recovery of mussels health two years after the Prestige oil spill. *Mar Environ Res.* 2006 Jul;62 Suppl:S337-41. Epub 2006 Apr 18.
- Christensen JH1, Tomasi G. Practical aspects of chemometrics for oil spill fingerprinting. *J Chromatogr A.* 2007 Oct 26;1169(1-2):1-22. Epub 2007 Sep 6.
- Conan, G. and Friha, M. 1979. Impact of hydrocarbon pollution from the 'Amoco- Cadiz' on the growth of sole and plaice in the inlets of Northern Brittany. *Cons. Int. Explor. Mer (ICES)*, E:54, 22pp.
- Crowe KM1, Newton JC, Kaltenboeck B, Johnson C. 2013. Oxidative stress responses of gulf killifish exposed to hydrocarbons from the Deepwater Horizon oil spill: Potential implications for aquatic food resources. *Environ Toxicol Chem.* 2014 Feb;33(2):370-4. doi: 10.1002/etc.2427. Epub 2013 Dec 12.

- Da Silva Rocha AJ1, Gomes V, Rocha Passos MJ, Hasue FM, Alves Santos TC, Bícago MC, Taniguchi S, Van Ngan P. EROD activity and genotoxicity in the seabob shrimp *Xiphopenaeus kroyeri* exposed to benzo[a]pyrene (BaP) concentrations. *Environ Toxicol Pharmacol*. 2012 Nov;34(3):995-1003. doi: 10.1016/j.etap.2012.07.006. Epub 2012 Jul 31.
- Desaunay, Y. (1981). Development of flatfish stocks in the zone contaminated by the 'Amoco-Cadiz'. Amoco-Cadiz, Actes Coll. Int. Centre Odanol. Bret., Brest, 19-22 November 1979. CNEXO Paris: 727-735
- de Maagd, G.-J., and A.D. Vethaak. 1998. Biotransformation of PAHs and Their Carcinogenic Effects in Fish. In Neilson, A. (Ed.). *The Handbook of Environmental Chemistry, Part 3 / 3J: Anthropogenic Compounds/PAHs and Related Compounds*. Springer-Verlag., Berlin, 386 pp
- Dubansky B1, Whitehead A, Miller JT, Rice CD, Galvez F. 2013. Multitissue molecular, genomic, and developmental effects of the Deepwater Horizon oil spill on resident Gulf killifish (*Fundulus grandis*). *Environ Sci Technol*. 2013 May 21;47(10):5074-82. doi: 10.1021/es400458p. Epub 2013 May 9.
- Debruyne, A. M. H., Wernick, B. G., Stefura, C., McDonald, B. G., Rudolph, B.-L., Patterson, L., & Chapman, P. M. (2007). In situ experimental assessment of lake whitefish development following a freshwater oil spill. *Environmental science & technology*, 41(20), 6983–9.
- Easton, R. (1972). *Black Tide: The Santa Barbara Oil Spill and its Consequences*. Delacorte Press.
- Edwards, R., and I. White (1999) *The Sea Empress Oil Spill: Environmental Impact and Recovery*. International Oil Spill Conference Proceedings: March 1999, Vol. 1999, No. 1, pp. 97-102.
- Eide M, Karlsen OA, Kryvi H, Olsvik PA, Goksøyr A 2013. Precision-cut liver slices of Atlantic cod (*Gadus morhua*): An in vitro system for studying the effects of environmental contaminants. *Aquat Toxicol*. 2013 Nov 2. pii: S0166-445X(13)00297-X. doi: 10.1016/j.aquatox.2013.10.027.
- Forsberg, N.D., B. Smith, G. Sower, and K. Anderson. 2014. Predicting Polycyclic Aromatic Hydrocarbon Concentrations in Resident Aquatic Organisms Using Passive Samplers and Partial Least-Squares Calibration *Environ. Sci. Technol.*, 2014, 48 (11), pp 6291–6299
- French-McCay, D. 2009. State-of-the-Art and Research Needs for Oil Spill Impact Assessment Modeling Proceedings of the 32nd AMOP Technical Seminar on Environmental Contamination and Response, Emergencies Science Division, Environment Canada, Ottawa, ON, Canada, pp. 601-653, 2009.
- Fernández-Tajes J, Rábade T, Laffon B, Méndez J. 2011. Monitoring follow up of two areas affected by the Prestige oil four years after the spillage. *J Toxicol Environ Health A*. 2011;74(15-16):1067-75. doi: 10.1080/15287394.2011.582312.
- Gallagher, J.J., and Nancy A. Gudonis (2008) UNIQUE LOGISTICS DIFFICULTIES ENCOUNTERED DURING RESPONSE TO THE M/V SELENDANG AYU STRANDING AND BREAK UP. International Oil Spill Conference Proceedings: May 2008, Vol. 2008, No. 1, pp. 1175-1183.
- Garmendia L1, Soto M, Vicario U, Kim Y, Cajaraville MP, Marigómez I. Application of a battery of biomarkers in mussel digestive gland to assess long-term effects of the Prestige oil spill in Galicia and Bay of Biscay: tissue-level biomarkers and histopathology. *J Environ Monit*. 2011 Apr;13(4):915-32. doi: 10.1039/c0em00410c. Epub 2011 Feb 3.
- Giari L1, Dezfouli BS, Lanzoni M, Castaldelli G. 2011 The impact of an oil spill on organs of bream *Abramis brama* in the Po River. *Ecotoxicol Environ Saf*. 2012 Mar;77:18-27. doi: 10.1016/j.ecoenv.2011.10.014. Epub 2011 Oct 24.

- S. D. Gill, C. A. Bonke, and J. Carter (1985) MANAGEMENT OF THE UNIACKE G-72 INCIDENT. International Oil Spill Conference Proceedings: February 1985, Vol. 1985, No. 1, pp. 311-313.
- Giari L1, Dezfuli BS, Canzone M, Castaldelli G. The impact of an oil spill on organs of bream *Abramis brama* in the Po River. *Ecotoxicol Environ Saf.* 2012 Mar;77:18-27. doi: 10.1016/j.ecoenv.2011.10.014. Epub 2011 Oct 24.
- Garmendia L1, Soto M, Vicario U, Kim Y, Cajaraville MP, Marigómez I. Application of a battery of biomarkers in mussel digestive gland to assess long-term effects of the Prestige oil spill in Galicia and Bay of Biscay: tissue-level biomarkers and histopathology. *J Environ Monit.* 2011 Apr;13(4):915-32. doi: 10.1039/c0em00410c. Epub 2011 Feb 3.
- Giari L1, Dezfuli BS, Lanzoni M, Castaldelli G. 2011 The impact of an oil spill on organs of bream *Abramis brama* in the Po River. *Ecotoxicol Environ Saf.* 2012 Mar;77:18-27. doi: 10.1016/j.ecoenv.2011.10.014. Epub 2011 Oct 24.
- Geraci JR and StAubinJ (eds)(1988) Synthesis of effects of oil on marine mammals. Atlantic OCS region: Minerals Management Service OCS 88-049
- GOGOASE-NISTORAN1,D.E., Delia-Mihaela POPESCU2, Valeriu PANAITESCU USE OF HYDRAULIC MODELING FOR RIVER OIL SPILLS.1. TRAVEL TIME COMPUTATION FOR QUICK RESPONSE U.P.B. Sci. Bull., Series D, Vol. 70, No. 4, 2008 ISSN 1454-2358
- Gulec, I. and D. Holdway. 1999. The Toxicity of Laboratory Burned Oil to the Amphipod *Allorchestes compressa* and the Snail *Polinices conicus*. *Spill Sci. Tech. Bull.* 5(2): 135-139.
- W. E. HAENSLY*, J. M. NEFF†, J. R. SHARP‡, A. C. MORRIS, M. F. BEDGOOD and P. D. BOEM‡ Histopathology of *Pleuronectes platessa* L. from Aber Wrach and Aber Benoit, Brittany, France: long-term effects of the Amoco Cadiz crude oil spill *Journal of Fish Diseases* Volume 5, Issue 5, pages 365–391, September 1982
- Harvey, J.S., B.P. Lyons, T.S. Page, C. Stewart, J.M. Parry. 1999. An assessment of the genotoxic impact of the Sea Empress oil spill by the measurement of DNA adduct levels in selected invertebrate and vertebrate species *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* Volume 441, Issue 1, 26 April 1999, Pages 103–114
- Helton, D., and Daniel Doty 2003 Emergency and Long-Term Restoration Planning for The 1999 Olympic Pipe Line Company Spill, whatcom Creek, Bellingham, Washington, USA International Oil Spill Conference Proceedings Apr 2003, Vol. 2003, No. 1 (April 2003) pp. 129-133
- Hinton D. E., Segner H., Braunbeck T. (2001). Toxic responses of the liver. In *Target Organ Toxicity in Marine and Freshwater Teleosts* (Schlenk D., Benson W. H., eds.), pp. 224–68. Taylor & Francis, London.
- Huggett, R. J.; Stegeman, J. J.; Page, D. S.; Parker, K. R.; Woodin, B.; Brown, J. S. 2003 Biomarkers in fish from Prince William Sound and the Gulf of Alaska: 1999-2000 *Environmental Science & Technology* 37(18): 4043-4051
- Husky Energy. 2010. White Rose Environmental Effect Monitoring (EEM) Program – 2010 Report EC-FT-00012. Submitted by Husky Energy to Canada-Newfoundland and Labrador Offshore Petroleum Board, St. John's, NL, 2010.
- Haung, T.S. (2011) The Montara Oil Spill Dispersant Response; Challenging the Paradigms. International Oil Spill Conference Proceedings: March 2011, Vol. 2011, No. 1, pp. abs 430.
- Huggett, R. J.; Stegeman, J. J.; Page, D. S.; Parker, K. R.; Woodin, B.; Brown, J. S. 2003 Biomarkers in fish from Prince William Sound and the Gulf of Alaska: 1999-2000 *Environmental Science & Technology* 37(18): 4043-4051

- IMO. 1998. IMO guidelines for sampling and identification of oil spills. Manual on oil pollution, Section VI. 44pp. London, UK. ISBN 978-92-801-1451-5.
- International Tanker Owners Pollution Federation (ITOPF). No date. Aerial observation of marine oil spills. ITOPF Technical Information Paper #1.
- International Tanker Owners Pollution Federation (ITOPF). No date. Recognition of oil on shorelines. ITOPF Technical Information Paper.
- International Tanker Owners Pollution Federation (ITOPF). No date. Leadership, command & management of oil spills. ITOPF Technical Information Paper.
- International Tanker Owners Pollution Federation (ITOPF). No date. Effects of oil pollution on fisheries and mariculture. ITOPF Technical Information Paper.
- Jung JH1, Kim M, Yim UH, Ha SY, An JG, Won JH, Han GM, Kim NS, Addison RF, Shim WJ. Biomarker responses in pelagic and benthic fish over 1 year following the Hebei Spirit oil spill (Taeon, Korea). *Mar Pollut Bull.* 2011 Aug;62(8):1859-66. doi: 10.1016/j.marpolbul.2011.04.045. Epub 2011 Jun 11.
- Jung, J-H. Won Joon Shim 2011 Biomarker responses in pelagic and benthic fish over 1 year following the Hebei Spirit oil spill (Taeon, Korea) *Marine Pollution Bulletin* Volume 62, Issue 8, August 2011, Pages 1859–1866
- Karl, DM 1992 The Grounding of the Bahia Paraiso: Microbial Ecology of the 1989 Antarctic Oil Spill *Microb Ecol* (1992) 24:77-89
- Kelly, C.A., R.J. Law and H.S. Emerson. 2000. METHODS FOR ANALYSIS FOR HYDROCARBONS AND POLYCYCLIC AROMATIC HYDROCARBONS (PAH) IN MARINE SAMPLES. *Sci. Ser., Aquat. Environ. Prot.: Analyt. Meth., CEFAS, Lowestoft*, (12), 18pp.
- Kirby, M. et al. 2000. Biomarkers of Polycyclic aromatic hydrocarbon exposure in fish and their application in marine monitoring. Centre for Environment, Fisheries and Aquaculture Science, Science Series Technical Report No. 10. 30 pp.
- Kirby, M., P. Neall*, T. Tylor. 1999. EROD activity measured in flatfish from the area of the Sea Empress oil spill *Chemosphere* Volume 38, Issue 12, May 1999, Pages 2929–2949
- Kocan, R. M., G. D. Marty, M. S. Okihira, E. D. Brown, and T. T. Baker. 1996b. Reproductive success and histopathology of individual Prince William Sound Pacific herring 3 years after the Exxon Valdez oil spill. *an. J. Fish. Aquat. Sci.* 53: 2388-2393.
- Kirby, M., Rosalinda Gioia, Robin J. Law. 2014. The principles of effective post-spill environmental monitoring in marine environments and their application to preparedness assessment *Marine Pollution Bulletin* 82 (2014) 11–18
- Kirby, M., P. Neall*, T. Tylor. 1999. EROD activity measured in flatfish from the area of the Sea Empress oil spill *Chemosphere* Volume 38, Issue 12, May 1999, Pages 2929–2949 (
- Kingston, P. 1999. Recovery of the Marine Environment Following the Braer Spill, Shetland. *International Oil Spill Conference Proceedings: March 1999, Vol. 1999, No. 1*, pp. 103-109.
- Law, R.J., Kirby, M.F., Moore, J., Barry, J., Sapp, M. and Balaam, J., 2011. *PREMIAM – Pollution Response in Emergencies Marine Impact Assessment and Monitoring: Post-incident monitoring guidelines. Science Series Technical Report, Cefas, Lowestoft*, 146: 164pp.
- Law, R.J., and J. Hellou 1999. Contamination of fish and shellfish following oil spill incidents *Environmental Geosciences*, v. 6, p. 90-98

- Law, R.J., Kirby, M.F., Moore, J., Barry, J., Sapp, M. and Balaam, J., 2011. **PREMIAM – Pollution Response in Emergencies Marine Impact Assessment and Monitoring: Post-incident monitoring guidelines**. Science Series Technical Report, Cefas, Lowestoft, 146: 164pp.
- Lee RF1, Anderson JW. 2005 Significance of cytochrome P450 system responses and levels of bile fluorescent aromatic compounds in marine wildlife following oil spills. *Mar Pollut Bull.* 2005 Jul;50(7):705-23.
- Leglise, M. and G. Raguene. 1981. Study of the effect of the 'Amoco-Cadiz' shipwreck on commercially exploitable crustaceans in the polluted zone. Amoco-Cadiz, Actes Call. Int. Centre Oceanol. Bret., Brest, 19-22 November 1979. CNEOX Paris: 775-787
- Marigómez I1, Soto M, Cancio I, Orbea A, Garmendia L, Cajaraville MP. Cell and tissue biomarkers in mussel, and histopathology in hake and anchovy from Bay of Biscay after the Prestige oil spill (Monitoring Campaign 2003). *Mar Pollut Bull.* 2006;53(5-7):287-304. Epub 2005 Nov
- Mackie, P.R., R. Hardy, and K. Whittle. 1978. Preliminary assessment of the presence of oil in the ecosystem at Ekofisk after the blowout, April 22-30, 1977. *J. Fish. Res. Bd. Can.*, 35:544-551.
- Martin 1985
- Martín-Díaz ML1, Blasco J, Sales D, Delvalls TA. 2007 Biomarkers study for sediment quality assessment in Spanish ports using the crab *Carcinus maenas* and the clam *Ruditapes philippinarum*. *Arch Environ Contam Toxicol.* 2007 Jul;53(1):66-76. Epub 2007 May 11.
- Martinez-Go'mez, C., Vethaak, A. D., Hylland, K., Burgeot, T., Ko'hler, A., Lyons, B. P., Thain, J., Gubbins, M. J., and Davies, I. M. 2010. A guide to toxicity assessment and monitoring effects at lower levels of biological organization following marine oil spills in European waters. – *ICES Journal of Marine Science*, 67: 1105–1118. Martínez-Gómez, J.A. Campilloa, J. Benedictoa, B. Fernández, J. Valdésa, I. García, F. Sánchezb Monitoring biomarkers in fish (*Lepidorhombus boscii* and *Callionymus lyra*) from the northern Iberian shelf after the Prestige oil spill *Marine Pollution Bulletin* Volume 53, Issues 5–7, 2006, Pages 305–314
- Martínez-Gómez, C. B. Fernández, J. Valdésa, J.A. Campilloa, J. Benedictoa, F. Sánchezb, A.D. Vethaakc Evaluation of three-year monitoring with biomarkers in fish following the Prestige oil spill (N Spain) *Chemosphere* Volume 74, Issue 5, February 2009, Pages 613–620
- Marty, GD Mark S Okihiro, Evelyn D Brown, David Hanes, David E Hinton 1999. Histopathology of adult Pacific herring in Prince William Sound, Alaska, after the Exxon Valdez oil spill. *Canadian Journal of Fisheries and Aquatic Sciences*, 1999, 56(3): 419-426, 10.1139/f98-178
- Marigómez, I., M. Soto, I. Cancio, A. Orbea, L. Garmendia, M. Cajaraville. 2006) Cell and tissue biomarkers in mussel, and histopathology in hake and anchovy from Bay of Biscay after the Prestige oil spill *Marine Pollution Bulletin* 53 (2006) 287–304
- Marty GD1, Hoffmann A, Okihiro MS, Hepler K, Hanes D. 2003. Retrospective analysis: bile hydrocarbons and histopathology of demersal rockfish in Prince William Sound, Alaska, after the Exxon Valdez oil spill. *Mar Environ Res.* 2003 Dec;56(5):569-84.
- McDonald, S.J., James M. Brooks, Dan Wilkson, Terry L. Wade, and Thomas J. McDonald. 1991. The Effects of the Apex Barge Spill on the Fish of Galveston Bay. *Proceedings: Galveston Bay Characterization Workshop*. February 21-23, 1991
- McFarlin, K. M., R. A. Perkins, W. Gardiner, J. Word, and J. Word. 2011. Toxicity of Physically and Chemically Dispersed Oil to Selected Arctic Species. *International Oil Spill Conference Proceedings: March 2011, Vol. 2011, No. 1*, pp. abs149.

- Meador JP1, Stein JE, Reichert WL, Varanasi U. 1995. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. *Rev Environ Contam Toxicol.* 1995;143:79-165. Michel, J., Frank Csulak, Deborah French and Molly Sperduto NATURAL RESOURCE IMPACTS FROM THE NORTH CAPE OIL SPILL International Oil Spill Conference Proceedings Apr 1997, Vol. 1997, No. 1 (April 1997) pp. 841-850.
- Moreira SM1, Moreira-Santos M, Ribeiro R, Guilhermino L. 2004. The 'Coral Bulker' fuel oil spill on the north coast of Portugal: spatial and temporal biomarker responses in *Mytilus galloprovincialis*. *Ecotoxicology.* 2004 Oct;13(7):619-30.
- Miossec, L. (1981a). Effects of the 'Amoco-Cadiz' pollution on the morphology and reproduction of plaice (*Pleuronectes platessa*) in the Wrach and Benoit estuaries; primary results. Amoco-Cadiz, Actes Coll., Int. Centre Oceanol. Bret., Brest, 19-22 November 1979. CNEXO Paris: 737-747
- Moreira SM, Moreira-Santos M, Ribeiro R, Guilhermino L. 2004. The 'Coral Bulker' fuel oil spill on the north coast of Portugal: spatial and temporal biomarker responses in *Mytilus galloprovincialis*. *Ecotoxicology* 13(7):619-30.
- Morales-Caselles C, Martín-Díaz ML, Riba I, Sarasquete C, DelValls TA. Sublethal responses in caged organisms exposed to sediments affected by oil spills. *Chemosphere.* 2008 Jun;72(5):819-25
- Moles, A., , Brenda L Norcross Effects of oil-laden sediments on growth and health of juvenile flatfishes *Canadian Journal of Fisheries and Aquatic Sciences*, 1998, 55:605-610, 10.1139/f97-278
- Martinelli, M., A. Luise, E. Tromellini, T. Sauer, J. Neff and G. Douglas. 1995. THE M/C HAVEN OIL SPILL: ENVIRONMENTAL ASSESSMENT OF EXPOSURE PATHWAYS AND RESOURCE INJURY. *International Oil Spill Conference Proceedings* 1995, pp. 679-685.
- Martínez-Gómez, J.A. Campilloa, J. Benedictoa, B. Fernández, J. Valdésa, I. Garcíaa, F. Sánchezb Monitoring biomarkers in fish (*Lepidorhombus bosci* and *Callionymus lyra*) from the northern Iberian shelf after the Prestige oil spill *Marine Pollution Bulletin* Volume 53, Issues 5-7, 2006, Pages 305-314
- Martínez-Gómez, C. B. Fernández, J. Valdésa, J.A. Campilloa, J. Benedictoa, F. Sánchezb, A.D. Vethaakc Evaluation of three-year monitoring with biomarkers in fish following the Prestige oil spill (N Spain) *Chemosphere* Volume 74, Issue 5, February 2009, Pages 613-620
- Marty GD1, Hoffmann A, Okihiro MS, Hepler K, Hanes D. Retrospective analysis: bile hydrocarbons and histopathology of demersal rockfish in Prince William Sound, Alaska, after the Exxon Valdez oil spill. *Mar Environ Res.* 2003 Dec;56(5):569-84.
- Martín-Díaz ML1, Blasco J, Sales D, Delvalls TA. 2007 Biomarkers study for sediment quality assessment in Spanish ports using the crab *Carcinus maenas* and the clam *Ruditapes philippinarum*. *Arch Environ Contam Toxicol.* 2007 Jul;53(1):66-76. Epub 2007 May 11.
- Marigómez I1, Soto M, Cancio I, Orbea A, Garmendia L, Cajaraville MP. Cell and tissue biomarkers in mussel, and histopathology in hake and anchovy from Bay of Biscay after the Prestige oil spill (Monitoring Campaign 2003). *Mar Pollut Bull.* 2006;53(5-7):287-304. Epub 2005 Nov
- Martinelli, M. Anna Luise, Elisabetta Tromellini, Theodor C. Sauer, Jerry M. Neff, and Gregory S. Douglas (1995) THE M/C HAVEN OIL SPILL: ENVIRONMENTAL ASSESSMENT OF EXPOSURE PATHWAYS AND RESOURCE INJURY. *International Oil Spill Conference Proceedings: February 1995, Vol. 1995, No. 1, pp. 679-685.*
- Michel, J., Frank Csulak, Deborah French, and Molly Sperduto (1997) NATURAL RESOURCE IMPACTS FROM THE NORTH CAPE OIL SPILL. *International Oil Spill Conference Proceedings: April 1997, Vol. 1997, No. 1, pp. 841-850.*

- Morales-Caselles, C, Jiménez-Tenorio N, de Canales ML, Sarasquete C, DelValls TA. 2006. Ecotoxicity of sediments contaminated by the oil spill associated with the tanker "Prestige" using juveniles of the fish *Sparus aurata*. Arch Environ Contam Toxicol. 2006 Nov;51(4):652-60. Epub 2006 Jun 20.
- Morales-Caselles C, Martín-Díaz ML, Riba I, Sarasquete C, DelValls TA. Sublethal responses in caged organisms exposed to sediments affected by oil spills. Chemosphere. 2008 Jun;72(5):819-25
- NATIONAL ENERGY BOARD OFFICE NATIONAL DE L'ÉNERGIE 2006. Weekly Status of Oil and Gas Activities on Frontier Lands Rapport hebdomadaire des activités pétrolières et gazières des régions pionnières Drilling Activities/Activités de forage As of 10 January 2006
- O'halloran, K., J. Ahorkas, P. Wright. 1998. The adverse effects of aquatic contaminants on fish immune responses. Australian Journal of Ecotoxicology 4:9-28.
- Page DS1, Huggett RJ, Stegeman JJ, Parker KR, Woodin B, Brown JS, Bence AE. Polycyclic aromatic hydrocarbon sources related to biomarker levels in fish from Prince William Sound and the Gulf of Alaska. Environ Sci Technol. 2004 Oct 1;38(19):4928-36.
- Payne, J.F., Fancey, L.L., Rahimtula, A.D., Porter, E.L., 1987. Review and perspective on the use of mixed-function oxygenase enzymes in biological monitoring. Comp. Biochem. Physiol. 6C, 233-245.
- Peteiro, L., J. Babarro, U. Labarta and M. J. Fernández-Reiriz. 2006. *Growth of *Mytilus galloprovincialis* after the Prestige oil spill ICES J. Mar. Sci. (2006) 63 (6): 1005-1013.
- Peterson, C.H., 1*Stanley D. Rice, 2Jeffrey Short, Daniel Esler, 3James L. Bodkin, 4Brenda E. Ballachey, 4David B. Irons. 2003. Long-term Ecosystem Response to the Exxon Valdez Oil Spill. Science 303 2082-2086.
- Page DS1, Huggett RJ, Stegeman JJ, Parker KR, Woodin B, Brown JS, Bence AE. Polycyclic aromatic hydrocarbon sources related to biomarker levels in fish from Prince William Sound and the Gulf of Alaska. Environ Sci Technol. 2004 Oct 1;38(19):4928-36.
- Perry, R. (1995) THE BRAER OIL SPILL. International Oil Spill Conference Proceedings: February 1995, Vol. 1995, No. 1, pp. 874-876.
- Rumney, H., Laruelle F, Potter K, Mellor PK, Law RJ. Polycyclic aromatic hydrocarbons in commercial fish and lobsters from the coastal waters of Madagascar following an oil spill in August 2009. Mar Pollut Bull. 2011 Dec;62(12):2859-62. doi: 10.1016/j.marpolbul.2011.09.019. Epub 2011 Oct 20.
- Reynaud S., and P. Deschaux. 2006. The effects of polycyclic aromatic hydrocarbons on the immune system of fish: A review. Aquat. Toxicol 77, 229-38.
- Rumney HS1, Laruelle F, Potter K, Mellor PK, Law RJ. Polycyclic aromatic hydrocarbons in commercial fish and lobsters from the coastal waters of Madagascar following an oil spill in August 2009. Mar Pollut Bull. 2011 Dec;62(12):2859-62. doi: 10.1016/j.marpolbul.2011.09.019. Epub 2011 Oct 20.
- S.L. Ross Environmental Research Ltd. 2002. SOEI Environmental Effects Monitoring Plan for Marine Spills (Sable Offshore Energy Inc., August 2002)
- S.L. Ross Environmental Research Ltd. 2002. Environmental Effects Monitoring Plan for Accidental Spills from Sable Offshore Energy Incorporated Facilities (Sable Offshore Energy Incorporated, February 2002)
- S.L. Ross Environmental Research Ltd. 2000. A Three-Tiered System for Implementing Environmental Effects Monitoring for Marine Oil Spills in Atlantic Canada (Canadian Association of Petroleum Producers and Sable Offshore Energy Incorporated, October 2000)

POPULATIONS ALONG LOWER TEXAS COAST BARRIER ISLAND BEACHES.

International Oil Spill Conference Proceedings: March 1981, Vol. 1981, No. 1, pp. 467-475.

Transportation Safety Board of Canada (TSBC). 2005. DERAILMENT CANADIAN NATIONAL FREIGHT TRAIN M30351-03 MILE 49.4, EDSON SUBDIVISION WABAMUN, ALBERTA. RAILWAY INVESTIGATION REPORT R05E0059, AUGUST 2005

Trudel 1985

Turcotte, D. 2008. TOXICITY AND METABOLISM OF ALKYL-POLYCYCLIC AROMATIC HYDROCARBONS IN FISH. Ph.D. Dissertation, to t Department of Chemistry Queen's University Kingston, Ontario, Canada

Vignier, V., J.H. Vandermeulen, J.Angus J. 1992. Growth and Food Conversion by Atlantic Salmon Parr during 40 Days' Exposure to Crude Oil. Transactions of the American Fisheries Society Volume 121, Issue 3, 1992

Viñas L1, Franco MA, Soriano JA, González JJ, Ortiz L, Bayona JM, Albaigés J. Accumulation trends of petroleum hydrocarbons in commercial shellfish from the Galician coast (NW Spain) affected by the Prestige oil spill. Chemosphere. 2009 Apr;75(4):534-41. doi: 10.1016/j.chemosphere.2008.12.003. Epub 2009 Jan 17.

van der Oost, R., Beyer, J., Vermeulen, N.P. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol. 2003 Feb;13(2):57-149.

Viñas L1, Franco MA, Soriano JA, González JJ, Ortiz L, Bayona JM, Albaigés J. Accumulation trends of petroleum hydrocarbons in commercial shellfish from the Galician coast (NW Spain) affected by the Prestige oil spill. Chemosphere. 2009 Apr;75(4):534-41. doi: 10.1016/j.chemosphere.2008.12.003. Epub 2009 Jan 17.

Wang Z, Yang C, Yang Z, Sun J, Hollebone B, Brown C, Landriault M. Forensic fingerprinting and source identification of the 2009 Sarnia (Ontario) oil spill. J Environ Monit. 2011 Nov;13(11):3004-17. doi: 10.1039/c1em10620a. Epub 2011 Sep 28.

Wang Z, Fingas M, Lambert P, Zeng G, Yang C, Hollebone B. Characterization and identification of the Detroit River mystery oil spill (2002). J Chromatogr A. 2004 Jun 4;1038(1-2):201-14.

Wiens, J., Ernest L. Brannon, David L. Garshelis, John Burns, Anne A. Hoover-Miller, Robert H. Day, Charles B. Johnson, and Stephen M. Murphy (1999) Fish and Wildlife Recovery Following the Exxon Valdez Oil Spill. International Oil Spill Conference Proceedings: March 1999, Vol. 1999, No. 1, pp. 127-133.

WSP Canada Inc., 2014. Risk Assessment for Marine Spills in Canadian Waters Phase 1, Oil Spills South of the 60th Parallel Final Version. Prepared for Transport Canada

Wang Z, Fingas M, Lambert P, Zeng G, Yang C, Hollebone B. Characterization and identification of the Detroit River mystery oil spill (2002). J Chromatogr A. 2004 Jun 4;1038(1-2):201-14. Wang, Z., S. A. Stout and M. Fingas. 2006. Forensic Fingerprinting of Biomarkers for Oil Spill Characterization and Source Identification Environmental Forensics 7:105-146, 2006

Wang Z, Yang C, Yang Z, Sun J, Hollebone B, Brown C, Landriault M. Forensic fingerprinting and source identification of the 2009 Sarnia (Ontario) oil spill. J Environ Monit. 2011 Nov;13(11):3004-17. doi: 10.1039/c1em10620a. Epub 2011 Sep 28.

Wells P.G. 1972. Influence of Venezuelan crude oil on lobster larvae Marine Pollution Bulletin Volume 3, Issue 7, July 1972, Pages 105-106

Whitehead A, Dubansky B, Bodinier C, Garcia TI, Miles S, Pilley C, Raghunathan V, Roach JL, Walker N, Walter RB, Rice CD, Galvez F. Genomic and physiological footprint of the Deepwater

Horizon oil spill on resident marsh fishes. Proc Natl Acad Sci U S A. 2012 Dec
11;109(50):20298-302. doi: 10.1073/pnas.1109545108

- Yasser M. Moustafa and Rania E. Morsi (2012). Biomarkers, Chromatography and Its Applications, Dr. Sasikumar Dhanarasu (Ed.), ISBN: 978-953-51-0357-8, InTech, DOI: 10.5772/35750. Available from: <http://www.intechopen.com/books/chromatography-and-its-applications/biomarkers>
- Yender, R., Michel, J. and Lord, C. 2002. Managing seafood safety after an oil spill. Hazardous Materials Response Division, Office of Response and Restoration. National Oceanic and Atmospheric Administration, Seattle, WA, USA. 72pp.
- Yender, R., Michel, J. and Lord, C. 2002. Managing seafood safety after an oil spill. Hazardous Materials Response Division, Office of Response and Restoration. National Oceanic and Atmospheric Administration, Seattle, WA, USA. 72pp.
- Zuckerman, S. (1967). The Torrey Canyon. Report of the Committee of Scientists on the Scientific and Technological Aspects of the Torrey Canyon Disaster. Departments of State and Official Bodies. Cabinet Office, London, UK

Appendix 1. Persistent and Non-Persistent Oils: What You Need To Know

By Caryn Anderson,
The International Tanker Owners Pollution Federation Limited (ITOPF)
Article in: "Beacon" (Skuld Newsletter) July 2001

Background

Marine oil spills have the potential to cause serious impacts to natural resources and the livelihoods that depend on them. The extent of impact however is influenced by a number of factors such as the type and amount of oil spilled, the physical characteristics of the affected area, the weather conditions at the time of the spill and the type and effectiveness of the response methods employed. The concept of persistence in relation to oil spills probably originated after the Torrey Canyon incident in 1967. This is the time when discussions first arose regarding various new measures to protect the marine environment and to manage marine oil spills, particularly in relation to liability and compensation. During this initial time, the primary concern following an oil pollution incident was in relation to cleanup and so, attention was focused on "black" oils that did not break down readily after a spill and because such oils 'persisted' long enough in the environment to warrant some form of response.

Generally, persistent oils do not dissipate quickly and will therefore pose potential threats to natural resources when released to the environment. Such threats have been evident in the past in terms of impacts to wildlife, smothering of habitats and oiling of amenity beaches. Cleanup techniques in response to persistent oils depend on the nature of the oil and the environment in which the oil has been spilled and include for example, the use of booms and skimmers for containment and recovery, the application of dispersants and manual cleanup of foreshores and coastlines. In contrast, when released to the environment, non-persistent oils will dissipate rapidly through evaporation. In light of this, spills of these oils rarely require a response but when they do, cleanup methods tend to be limited. Impacts from non-persistent oils may include, for example, effects on paint coatings in marinas and harbours and at high concentrations, acute toxicity to marine organisms.

Definition of Persistent Oils

As most are aware, both the 1969 Civil Liability Convention and the 1971 Fund Convention apply only to spills of "persistent" oil. The Conventions were initially drafted to cover costs of the removal of pollutants from the sea and shore. The concept of persistent and non-persistent oils related therefore to the likelihood of the material dissipating naturally at sea and whether or not cleanup would be required. However, a precise definition of persistent oil is not provided and interpretation has historically relied on the examples given in the Conventions such as crude oil, fuel oil, heavy diesel oil and lubricating oil. The lack of a precise definition led the International Oil Pollution Compensation (IOPC) Fund, as the administrator of the 1971 Fund Convention to seek to clarify the definition and to develop a working model for practical implementation. As a result, a study was commissioned to establish a working definition of persistent oils. The study based the distinction between persistent and non-persistent oils on the distillation characteristics of the oil shipped. Non-persistent oils are those that are generally of a volatile nature and are composed of lighter hydrocarbon fractions, which tend to dissipate rapidly through evaporation. In contrast, persistent oils generally contain a considerable proportion of heavy fractions or high-boiling material. In the definition adopted by the IOPC Fund, persistent oils are actually defined by describing what is meant by non-persistent oil:

"non-persistent oil is oil which, at the time of shipment, consists of hydrocarbon fractions,

- a) at least 50% of which, by volume, distils at a temperature of 340 °C (645°F); and
- b) at least 95% of which, by volume, distils at a temperature of 370 °C (700°F);

when tested by the ASTM Method D86/78 or any subsequent revision thereof”.

The boundary set by this definition might be considered to be somewhat arbitrary particularly given the continuous spectrum of oil types with varying degrees of persistence. The definition may also give rise to other difficulties. For example, it is interesting to note that the definition developed by the IOPC Fund cannot be applied to non-mineral oils (despite the physical persistence of some of these oils) because they cannot tolerate the distillation process. On the other hand, whilst the 1969 CLC applies to any type of persistent oil (including non-mineral oils such as whale oil), the definition of oil was revised in the 1971 Fund Convention and in the subsequent '92 CLC and FC to apply only to 'persistent hydrocarbon mineral oils'. Despite these concerns, it should be recognised that the definition adopted by the IOPC Fund does provide clear guidance on those oils that are covered by the Conventions. The term “persistent” and the chemical definition relied upon by the Conventions and those who apply them ensures consistency in the application of the term and overcomes the variety of terminologies that may be used on a local or regional basis. This consistency is very important in the context of the United States where under the Oil Pollution Act 1990 (US) even though the concept of persistent/non-persistent oil has no direct relevance in the law. However, given the significant potential liability associated with loading or discharging persistent oil cargoes in waters of the US, it has been necessary to apply a weighting on such voyages. The P&I Clubs have adopted the IOPC Fund definition of persistence/non-persistence as a convenient standard by which to apply an additional premium on persistent oil cargoes deemed to represent a greater risk of financial exposure in the event of oil pollution. Thus, the advice is often sought from ITOPF on the determination of the persistence or otherwise of an oil and to interpret the IOPC Fund definition. As described above, the assessment is based on the distillation characteristics of the individual oil.

Appendix 2. Method for Analysis of EROD Activity in Fish Liver Tissue

There are numerous published procedures for assaying EROD in fish gills and liver. The method listed below is reported in Richardson et al. 2001.

Richardson, D., I. M. Davies, C. F. Moffat, P. Pollard and R. M. Stagg. 2001. Biliary PAH metabolites and EROD activity in flounder (*Platichthys flesus*) from a contaminated estuarine environment J. Environ. Monit. 3:610-615

Partially defrosted liver samples were homogenised with a Potter-Elvehjem type homogeniser in an ice cold phosphate buffer containing 0.1 M K₂HPO₄-3H₂O, 0.1 M KH₂PO₄, 0.15 M KCl, 1mM EDTA, 0.1 mM dithiothreitol, pH 7.4. The homogenate was transferred into Tref-tubes and centrifuged at 10000g at 4°C for 20 min. The resultant supernatant was snap frozen and stored at — 70 °C until analysis.

EROD activity was determined according to the method of Burke and Mayer (24) as described by Stagg et al. (8) Resorufin production was measured in a temperature controlled (20 °C) cuvette with continuous stirring of 1960 µl 100 mM pH 7.4 phosphate assay buffer (0.1 M K₂HPO₄-3H₂O, 0.1 M KH₂PO₄, 0.15 M KCl). 7-Ethoxyresorufin (10 µl, 0.4 mM) in DMSO solution and a sample supernatant (20 µl) was then added. The reaction was started by the addition of NADPH (10 µl, 100 mM) and calibrated by the addition of an internal spike of resorufin (10 µl, 12.5 pM). Resorufin was normalised on a daily basis using the molar absorptivity of 73.2 mM⁻¹ cm⁻¹. The increase in fluorescence with time was measured over a 2 to 10 min period using a Perkin Elmer LS50B luminescence spectrometer and analysed with Enzyme Activity Rev 3.0 (Biolum Ltd) software. The excitation and emission wavelengths were 535 nm and 585 nm, respectively. Reaction rates were expressed as pmol of resorufin produced per min per mg of protein.

The protein content was determined colorimetrically according to the method of Lowry et al. (25) with bovine serum albumin (BSA) as the reference standard. The assay was performed using a Biorad Protein Assay kit, on an Argus plate reader at 595 nm. The reaction product was quantified against the response produced by the serial dilution (0.2-1.6 mg ml⁻¹) of the BSA standard.

Appendix 3. Method for Analysis of Glutathione-S-Transferase (GST) Activity in Fish Tissue

The methods listed below were reported in Rudneva et al 2010.

Rudneva, U., N., Kuzminova, E. Skuratovskaya. 2010. Glutathione-S-Transferase (GST) Activity in Tissues of Black Sea Fish Species. ASIAN J. EXP. BIOL. SCI., VOL1 (1)2010:141-150

Enzyme assays GST activity was determined by the method of Habig et al. (1974) by following the increase in absorbance at 340 nm due to the formation of the conjugate 1-chloro-2,4-dinitrobenzene (CDNB) using as substrate at the presence of reduced glutathione (GSH). The reaction mixture was prepared by mixing 1.5 ml sodium phosphate buffer 0.1 M pH 6.5, 0.2 ml GSH 9.2 mM, 0.02 ml CDNB 0.1 M and 0.1 ml of the sample. The absorbance was measured at 340 nm and at the temperature, +25°C spectrophotometrically using Specol-211 (Germany). The increase in absorbance was recorded for a total of 3 min. The reaction solution without the fish homogenates and blood lysates was used as blank. Enzymatic activity was calculated via the formula:

$$A = \frac{1000 \times (E_{exp} - E_{cont}) \times 1.82}{9.6 \times t \times c}$$

where A is enzyme activity, conjugate nmol/mg protein/min; E_{exp} is increase of the optical density at 340 nm of the sample; E_{cont} is increase of the optical density at 340 nm of the blank; 1000 is coefficient, 1.82 is the total volume of the mixture, ml; 9.6 is molar coefficient of the conjugate formation; V is volume of the sample, l; t-time, min; c-protein or hemoglobin (Hb) concentration. The protein concentration in the liver homogenates was estimated by the method of Lowry et al. (1951) using human serum albumin as the standard protein. Hemoglobin concentration in blood lysates was detected spectrophotometrically, using human hemoglobin as a standard concentration.

References Cited

Habig, W.H., Pabst, M.J. and Jokoby, W.B. (1974). Glutathione S-transferase. The first enzyme step in mercapturic acid formation. J. Biol. Chem., 249: 7130-139.
Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin-phenol reagent. J. Biol. Chem., 193:165-175.

Appendix 4. PAH Metabolites in Bile of Fish

There are numerous published procedures for analysing for PAH metabolites in bile in fish. The one listed below is reported in Richardson et al. (2001).

Richardson, D., I. M. Davies, C. F. Moffat, P. Pollard and R. M. Stagg. 2001. Biliary PAH metabolites and EROD activity in flounder (*Platichthys flesus*) from a contaminated estuarine environment J. Environ. Monitor 2001, 3, 610-615

Bile samples are hydrolysed by a modification of the method of Ariese et al. 18 Bile (20 μ l) is mixed with 200 μ l of HPLC grade water + 1% ascorbic acid to which 20 μ l of β -glucuronidase-arylsulfatase solution (5.5 and 2.6 U ml⁻¹, respectively) was added. The resultant solution was incubated for 1 h at 37 °C in a shaking water bath. The hydrolysed metabolites were diluted with ethanol-water (250 μ l, 84% ethanol by weight) containing 4% (by weight) ascorbic acid. The final solution was centrifuged (10000g) at 4°C for 5 min. One aliquot of 50 μ l of the solution was removed for HPLC-fluorescence analysis.

The concentration of the individual hydroxylated PAHs was determined using a Hewlett Packard series 1050 HPLC system comprising a quaternary pump, de-gas unit, and autosampler. Samples were chromatographed on a Vydac 201TP54 (25 cm x 4.6 id) C₁₈ reverse phase analytical column, non-endcapped with 0.5 μ m x 3 mm Phenomenex filter. The hydroxylated compounds were detected using a Waters 470 Scanning fluorescence detector. Injections (20 μ l) were made at 40 °C and the oven temperature remained constant throughout the run. The initial mobile phase was 20 : 80 v/v acetonitrile-water (water was acidified to pH 4 with acetic acid). The solvent composition progressively changed to 100% acetonitrile over 45 min. At the end of each run the column was allowed to reequilibrate over 5 min.

The excitation: emission-wavelength pairs (nm) for 2-OH naphthalene, 1-OH phenanthrene, 1-OH pyrene were 222-370, 246-370, 243-388, respectively. Bile samples were analysed individually and the results are expressed as the mean OH- metabolite (nmol per ml of bile, pSE) for each site.

References Cited

Ariese, F., S. J. Kok, M. Verkaik, C. Gooijer, N. H. Veithorst and W. Hoofstraat. 1993. Synchronous fluorescence spectrometry of fish bile: A rapid screening method for the biomonitoring of PAH exposure. Aquat. Toxicol., 1993, 26, 273.

Appendix 5. Method for Assessing Sections of Liver Tissues from Flatfish for Histopathology.

The methods listed below were reported in Marigómez et al. (2006).

Marigómez, I., M. Soto, I. Cancio, A. Orbea, L. Garmendia, M. Cajaraville. 2006. Cell and tissue biomarkers in mussel, and histopathology in hake and anchovy from Bay of Biscay after the Prestige oil spill Marine Pollution Bulletin 53: 287–304.

Histopathology of fish liver. - Liver paraffin sections (5µm) were stained with hematoxylin–eosin. The histopathological study was carried out by examining each sample individually under the light microscope. Histopathological examination was carried out according to the recommendations made by the BE-QUALM program (Biological Effects Quality Assurance in Monitoring Programs, Liver Histopathology and External Diseases Training CD-ROM), which indicate the criteria to identify the most outstanding pathological alterations in flat fishes (*Limanda limanda*, *Platichthys flexus*) from the ICES zone. Histopathological criteria were discussed with local experts. Prevalence of parasites, inflammatory changes (infiltration and granulomatosis), atrophy, necrosis, apoptosis and the presence of increased numbers and size of melanomacrophage centres (MMCs) were recorded and included in the category of non-specific lesions. Further four categories of pollution-related lesions were considered (Feist et al., 2004): Early non-neoplastic toxicopathic lesions (hepatocellular nuclear polymorphism, hydropic vacuolization, phospholipidosis and fibrillar inclusions), foci of cellular alteration (clear cell foci, vacuolized foci, eosinophilic foci, basophilic foci, mixed cell foci), benign neoplasms (hepatocellular adenoma, cholangioma, hemangioma, pancreatic acinar cell adenoma), and malignant neoplasms (carcinoma, sarcoma, etc.).